Report of the Haffkine Institute

For the Years 1940 and 1941

BY

LT.-COL. S. S. SOKHEY, I.M.S.

Director, Haffkine Institute

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REPORT OF THE HAFFKINE INSTITUTE FOR THE YEARS 1940 AND 1941

PART I

STAFF

DIRECTOR

Lt. Col. S. S. Sokhey, M.A., M.D. (Edin.), D.T.M.& H. (Eng.), F.A.S., F.N.I., I.M.S.

Vaccine Department

Lt. Col. R. C. Wats, M.D., Ch.B. (Liverpool), D.P.H., D.T.M., I.M.S., (From 1st January 1940 to 13th February 1941).

Dr. P. M. Wagle, M.D. (Bom.), D.Bac. (Manch.), D.P.H. (Lond.), D.T.M. (Liverpool), B.M.S.

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Mr. T. K. Puduval, B.A.

Mr. R. Sadashivan, B.Sc.

Mr. M. K. Habbu, B.Sc.

Antitoxin and Serum Department

Dr. D. C. Lahiri, M.B. (Cal.), D.T.M. & H. (Eng.), D.B. (Lond.), B.M.S.

Mr. R. G. Sathe, G.B.V.C.

Department of Pharmacology

Dr. B. B. Dikshit, Ph.D.(Edin.), M.R.C.P. (Edin.), D.P.H. (Cal.).

Dr. B. K. Nandi, Ph.D., M.Sc. (From 1st January 1940 to 31st October 1940).

Dr. H. S. Mahal, Ph.D., M.Sc., A.I.C.

Department of Biochemistry

Mr. M. A. Malandkar, M.A., M.Sc.

Mr. S. K. Gokhale, B.A., M.Sc., A.I.I.Sc.

Department of Chemotherapy

Dr. K. Ganapathi, D.Sc.

Mr. M. V. Shirsat, M.Sc.

Mr. C. V. Deliwala, M.Sc.

Department of Entomology

Dr. M. Sharif, D.Sc. (Pb.), Ph.D. (Cantab.), F.N.I., B.M.S.

Mr. A. S. Narasimham, B.A.

Department of Pathology

Dr. M. V. Radhakrishna Rao, M.B.B.S., Ph.D. (Andhra), B.M.S.

Mr. A. J. Naidu.

Diagnostic Department

Dr. D. W. Soman, M.B.B.S., B.Hy., D.P.H., D.T.M. & H., B.M.S.

Mr. G. S. Bhat, L.C.P.S., S.M.S.

Mr. G. K. Kamat, B.Sc.

Mr. R. B. M. Colah, M.Sc.

Antirabic Department

Dr. R. Sanjiva Rao, M.B.B.S. (Mad.), D.T.M. (Liv.), B.M.S.

Mr. R. K. Gokhale, L.C.P.S., S.M.S.

Plague Inquiry

Major S. S. Bhatnagar, M.B.B.S. (Pb.), M.R.C.P. (Lond.), M.R.C.S. (Eng.), Ph.D. (Lond.), I.M.S. (up to 14th October 1940).

Dr. D. L. Shrivastava, D.Sc. (up to 1st February 1941).

Dr. S. C. Seal, M.B.

Mr. S. P. Mukherji, M.Sc.

Leptospirosis Inquiry

Dr. M. N. Lahiri, M.B. (Cal.), D.T.M. (L'pool).

Scholars

Mr. M. Sadashiv Rao, M.A.

Mr. R. N. Kulkarni, M.Sc.

Dr. R. A. Joshi, M.B.B.S.

Mr. N. C. Datta, M.Sc.

Mr. S. Rajagopalan, M.Sc.

Mr. D. M. Munshi, B.Sc., F.R.M.S.

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GENERAL REVIEW

These two years have meant a period of strenuous effort to cope with the numerous problems presented by the War. The work of the Institute had to be considerably expanded under difficult conditions to give every possible aid to the Defence Services and the A.R.P. organisations and maintain at a high level the research activities of the Institute. The existing departments were expanded to meet increased demands for vaccine and to produce large quantities of solutions for intravenous and subcutaneous administration. The Pharmacological Department carried out chemical analyses and bio-assays of hundreds of samples of drugs, pharmacopoeal preparations and other medical supplies purchased by the Army in Western India or imported through the Port of Bombay. This service has helped the forces to maintain the quality of their medical supplies at a high level. Similar service is urgently needed for civilian agencies.

New Departments Organised

Two new departments were organised to prepare antitoxins and sera and some of the more important synthetic drugs for which the country was wholly or partially dependent on foreign sources and the supplies of which had been cut off or whittled down. Towards the end of 1940, the production of antitoxins and sera was decided upon. The foreign supplies were difficult to obtain and indigenous commercial production was small and was not controlled by any Government supervision. The Institute was fortunate in securing the services of Dr. Dhirendra Chandra Lahiri, M.B. (Cal.), D.T.M. & H. (Eng.), D.B. (Lond.) a worker with considerable experience of large scale serum production. Production on an experimental basis was started and this soon proved to be a great success. Tetanus antitoxin, Diphtheria antitoxin, Anti-dysentery serum, Anti-Gas gangrene serum, Tetanus and Diphtheria toxoids of the highest quality were produced in sufficient quantity to meet the needs of the province. This experimental production showed that these biological products could be produced at $\frac{1}{2}$ to $\frac{1}{3}$ the cost of imported products. This fact was brought to the notice of different provincial governments and medical administrations of almost all the provinces of India desired to be supplied by the Institute. Production on a large scale was immediately planned and construction of the necessary buildings taken in hand and the required equipment ordered. While organisation of the department is proceeding, every effort is being made to meet the demands of the civil hospitals, A.R.P. organisations all over the country as well as that of the Army. It is hoped it would be possible to fully equip this department during the coming year.

The second department to be organised was the Department of Chemotherapy. The recent phenomenal advances in the treatment of disease secured by synthetic chemistry made the organisation of a department of Chemotherapy at the Institute highly desirable. The Institute already had well developed departments of Bacteriology and

Pharmacology and was thus an ideal place for such development. Services of Dr. Krishnamurti Ganapathi, D.Sc., were secured and work commenced in 1940. Research in this field is the principal activity of this department. The department has produced excellent work in its short existence, among which it has to its credit the discovery of Sulphathiazole as an effective agent for the treatment of Bubonic Plague. But that is not all, very active research into new compounds has been carried out. A large number of derivatives of sulphanilamide radical and also compounds with various types of substituents in sulphathiazole have been synthesised. The therapeutic effects of 89 of these new compounds have been studied in experimental infection. Of these five compounds have shown pronounced therapeutic activity and their properties are being further studied in detail. Besides research the department has done valuable work in investigating into possible production on a semi-commercial scale of the more important synthetic drugs, should conditions demand it. The department has proved of very great value even during its short existence.

Researches

The researches carried out during the period under review are described in Part II of this report, but attention is drawn here to some of the more important findings except those of the chemotherapy studies which have been touched upon in the paragraph above.

Chemotherapy of Plague.—The results of three field trials conducted to study the comparative curative value of Sulphathiazole, Sulphapyridine, Haffkine Institute Antiplague Serum and Sulphathiazole cum Antiplague Serum are given in Part II of this report. In all 682 cases were treated. The results show that all the three therapeutic agents tested are possessed of marked curative action against Bubonic Plague. Statistical analysis of the results shows that " as compared with Sulphapyridine, Sulphathiazole produces a significantly higher recovery rate among cases with severe septicaemia, i.e. more than 10 colonies per 0·25 cc. of blood. " Best results seem to have been obtained when both Serum and Sulphathiazole were used together but the number of cases so treated was too small to make the observation conclusive.

Plague Vaccine.—Two interesting observations are reported which show (1) that the immunogenicity of a strain of plague culture varies with its virulence, and (2) that the method of killing the cultures affects the protective power of the resultant vaccine. These observations suggest the possibility of making a new type of plague vaccine, which besides being very highly potent would produce much less reaction, local and general, than the present Haffkine vaccine. Work is well advanced on this point.

Plague Recrudescence Inquiry.—The lack of understanding of the factors governing the persistence of plague in the Deccan Plateau has hampered the employment of effective antiplague measures. A field unit took up

the study of these factors in Sholapur and Dharwar Districts. A year and a half's work has yielded valuable information. Contrary to expectation wild rodents did not seem to play any part in carry-over of plague from one season to another or serve as reservoirs. Agent responsible for carry-over seems to be the flea, its behaviour towards climatic conditions being the deciding factor. During cool and wet weather fleas are abundant in rooms and can remain on the human body for a long period, whilst they go underground into the burrows during the hot and dry season. Thus during the hot weather infection is only confined to burrows and moves from burrow to burrow through the agency of wandering house rats without causing any serious epizootic or epidemic. With the onset of favourable weather the dispersal of infection takes place with the movements of grain from the villages to a neighbouring grain centre and from this to fresh villages. Because the grain centres have had epizootics over a long period of time, their rat population has become largely immune. Thus owing to the higher percentage of immune rats in grain centres, they almost appear to be free from plague, but in small villages where the rats are more susceptible, the infection flares up into severe epizootic leading to epidemics.

Epidemiology of Plague.—In the two previous annual reports 1937 and 1939, it was pointed out that the population of *Gunomys kok* in the Bombay City area was gradually increasing, and at present it forms about 40 per cent of the entire rodent population of the city, as compared with only one per cent in 1907. Apparently, this species is becoming more numerous and is tending to replace the domestic rats in human habitations. In view of findings of the Plague Recrudescence Inquiry in Sholapur and adjoining Districts that this species is more or less exclusively found in the fields and very rarely is found in human habitations in villages and small towns, it is suggested that this species is becoming domesticated in the Bombay Island. Owing to the continual acquisition of open space for habitation purposes, very little of the cultivated fields are left for these wild rodents. Consequently, they have been forced to depend upon man for food and shelter. This recently acquired close association with man by these wild rodents highly susceptible to plague is a point for a serious consideration, especially when plague still persists in some districts of this province, doing trade in grain with this city. Further, as pointed out in the last annual report that the percentage of susceptible house rats is also increasing in the city, these two facts lend a strong support to the assumption that the position of this city as regards the possible recrudescence of plague is worsening, and the stage has been set now for fresh epidemics.

Sulphonamides in Malarial Infection.—The situation created by the lack of anti-malarial drugs like quinine and atebrin and plasmoquine is undoubtedly serious and the Pharmacology Department of the Institute has endeavoured to find a drug which can be used as an anti-malarial drug instead of these well-known remedies. It was found that Sulphathiazole and another drug prepared by the Institute viz.

2-N^1-sulphanilamido-5-ethylthiazole produces a remarkable curative effect in monkey malaria. It is important to note that while quinine and atebrin produce a beneficial effect in monkey malaria, there is always a relapse of the infection within a fortnight. Treatment with the two sulphonamides mentioned above, however, is followed by complete eradication of infection from the monkeys. Our researches have, therefore, shown that the sulphonamides are not only active in bacterial infections but in protozoal infections as well. Cure of monkey malaria by a drug does not necessarily mean that the drug will be useful in human malaria also. The two sulphonamides are still to be tried in human malaria. It is, however, expected that further researches in this line may enable us to find a drug which will be useful in human malarial infections. Active research is proceeding.

Sulphathiazole in Burns and Wounds.—The treatment of burns has again assumed importance, specially as older methods were found to be wanting. In experimental burns of different degrees a suspension of sulphathiazole in cod-liver oil gave better results than other commonly employed methods of treatment. Similarly in crushed wounds, experimentally infected with septic organisms, application of Sulphathiazole powder produced remarkable results.

SECTION I

Vaccine Department

A.—*Haffkine's Plague Vaccine*

During the years, 1940 and 1941, 1,905,709 doses and 1,363,296 doses of plague vaccine respectively were issued against 962,509 doses in the year 1939.

The number of doses of plague vaccine despatched monthly during the years 1939, 1940 and 1941 are detailed below :—

——	1939	1940	1941
January	51,350	139,947	285,189
February	104,138	98,632	132,136
March	115,942	118,722	177,540
April	58,618	118,077	36,935
May	4,910	4,114	2,284
June	6,660	25,935	1,317
July	17,303	66,195	83,822
August	121,323	349,840	86,156
September	169,267	352,320	128,220
October	110,171	218,991	140,200
November	90,153	194,813	134,081
December	112,674	218,123	155,416
Total ..	962,509	1,905,709	1,363,296

From the opening of the Institute to the end of 1941, a total of 47,603,308 doses have been issued. The output of the vaccine in quinquennial periods has been as follows :—

Years	Doses
1897–1901	2,867,041
1902–1906	3,455,537
1907–1911	3,584,262
1912–1916	4,567,366
1917–1921	6,667,894
1922–1926	7,202,636
1927–1931	5,929,243
1932–1936	6,636,392
1937–1941	6,692,937
Total ..	47,603,308

The geographical distribution of the vaccine in India and outside India during the years 1939, 1940 and 1941 is given below :—

	Number of doses		
	1939	1940	1941
BRITISH INDIA			
Bombay Province ..	129,780	556,181	308,321
Madras Province ..	36,762	192,391	196,749
Bengal, Bihar and Assam ..	47,946	44,599	4,203
United Provinces of Agra and Oudh.	183,360	232,032	81,701
Punjab	2,294	1,145	1,431
North-West Frontier Province.	300	210	300
Central Provinces and Berar ..	41,786	28,624	54,462
Delhi Province ..	92	120	300
Sind Province	324	985	1,977
Port Blair	24	36	24
Total ..	442,668	1,056,323	649,468

	Number of doses		
	1939	1940	1941
INDIAN STATES			
Southern Maratha States ..	28,756	44,595	32,922
Hyderabad Deccan ..	226,800	274,808	166,047
Mysore	107,736	217,492	231,161
Patiala	30	66	204
Jaipur	47	....	72
Jodhpur	60	30	30
Indore	30	51	562
Cochin	....	25	48
Baroda	....	96	2,112
Gwalior	600	....	1,008
Junagad	1,800	....	....
Benares	5,157	504	600
Travancore	....	10,844	....
Rajputana	36	15	....
Panna	75	....	....
Tikamgarh	3,606	600	....
Nabha	....	200	....
Chhatarpur	....	504	....
Bhopal	....	....	144
Total ..	374,823	549,830	434,910
FOREIGN COUNTRIES			
Burma	143,052	117,936	171,360
Federated Malaya States ..	744	1,008	9,576
Egypt	1,200	180,000	60,480
Muskat, Arabia	20	....	....
Singapore	....	300	1,058
Portuguese Government ..	....	120	....
French Government ..	2	....	....
Aden	....	....	1,038
China	....	192	35,280
Iraq	....	....	126
Total ..	145,018	299,556	278,918
Total number of doses ..	962,509	1,905,709	1,363,296

B.—*Statement showing the number of cc. of anti-cholera and anti-typhoid vaccines issued during the years 1940 and 1941*

Months	Anti-cholera Vaccine in cc.		T. A. B. Vaccine in cc.	
	1940	1941	1940	1941
January ..	891	54,015	1,975	1,291
February ..	291	11,920	1,216	714
March ..	2,856	80,071	819	2,155
April ..	3,757	219,290	920	2,857
May ..	57,716	307,224	1,160	4,633
June ..	56,471	273,227	837	4,253
July ..	184,762	386,532	1,275	11,452
August ..	40,048	468,279	2,982	8,562
September ..	17,040	237,325	3,762	5,818
October ..	4,705	627,435	3,344	14,433
November ..	6,285	101,835	1,430	10,307
December ..	45,349	17,619	1,441	615
Total ..	420,171	2,784,772	21,161	67,090

SECTION II

Antitoxin and Serum Department

The work of this department started on the 1st of October 1940. By November enough of tetanus and diphtheria toxoids was produced to enable starting immunization of 27 horses. By February 1941, a few of the horses were taken up for production of Gas gangrene and Shiga antitoxins also. At first it was contemplated to meet the demands only of the Bombay Government Hospitals. By the middle of the year, however, it became clear that the scale of production must be expanded to extend to other Provincial Governments and Indian States facilities for getting their supplies from the Institute. As there was at that time no accommodation to house any more horses comfortably, recourse had to be had to hyperimmunize the horses by the rapid

method and to bleed them as often as possible. In October 1941, a new block of 18 stables and a special isolated block for preparation of tetanus and gas gangrene toxins were got ready and 18 more horses were obtained. The A. R. P. authorities of different provinces were at that time asking the Institute for their supply of essential sera, each item amounting to tens of thousands of doses. It was, therefore, necessary that the maximum production of antitoxin possible with such a limited number of horses must be obtained. Still it was doubtful whether even such a tremendous effort could meet such huge demands. It was with this anxiety in the minds of the workers that the year came to a close.

The following amounts of different toxins were produced during the period under review :—

(*a*) Tetanus Toxin	.. 65 batches, 4 to 12 litres each.
(*b*) Diphtheria Toxin	.. 32 batches, 2 to 10 litres each.
(*c*) Gas gangrene (*Cl Welchii*, *Cl septique* and *Cl. oedematines*) Toxins	.. 15 batches, 1 to 5 litres each.
(*d*) Shiga Toxin	.. 5 batches, 12 litres each.

During the year 1941, 14 batches of Tetanus Toxoid were prepared specially for human use. Eleven of them passed the tests laid down by the British Therapeutic Substances Regulations. Some of them were issued to different authorities for use in man. A part of the different batches of toxoid was used for immunizing the laboratory and the stable personnel divided into random groups. The antitoxin titre of their sera were determined in terms of International units. The figures obtained compared very favourably with those published by workers abroad.

Experiments were also done to evolve out a test for assaying tetanus toxoid less expensive in time and animals than that laid down by the British Therapeutic Substances Regulations. As a result of these experiments a mouse protection test was evolved which can be used for preliminary assay of different batches of tetanus toxoid.

Two batches of Diphtheria Toxoid, 2 litres each, were also produced during the year.

During the period under review, 56 batches of different antitoxins were prepared. Most of them were concentrated and purified. The volume of plasma or serum in each batch amounted from 10 to 25 litres.

During 1940 and first nine months of 1941, sera obtained in bulk from foreign firms and bottled at the Institute were continued to be issued in order to use up the existing stock. These amounted to 24,787

ampoules. During the last three months of 1941, the following numbers of ampoules of different amounts of various antitoxins prepared at the Institute were issued :—

Tetanus antitoxin, 20,000 I. U., 10 cc. ..	1,009	ampoules.
Tetanus antitoxin, 3,000 I. U., 2 cc. ..	13,179	,,
Tetanus toxoid 1 cc. each	800	,,
Anti-dysentery serum, polyvalent, natural, in a volume of 20 cc.	100	,,
Diphtheria antitoxin, 10,000 I. U., 10 cc. ..	133	,,
Diphtheria antitoxin, 1,000 I. U., 2 cc. ..	44	,,
Anti-Gas gangrene serum, 3,000 I. U. Welchii, 1,500 I. U. septique and 1,000 I. U. oedematiens antitoxins, in a volume of 10 cc. each	253	,,

SECTION III

Department of Entomology and Epidemiology of Plague

This department carried out a number of routine duties and researches during the two years. Investigations on the effects of different constant temperatures and humidities on the rate of growth of early stages of the three Indian rat-fleas and on the nutritional requirements of their larvae were concluded. The results of these investigations are summarized in Part II of this report and will be shortly published.

In addition the department was also engaged in organizing and conducting an inquiry into the recrudescence of plague in Sholapur and adjoining districts sanctioned by the Government of Bombay.

The object of this inquiry is to investigate the causes of plague recrudescence in the Deccan Plateau. It is a well-known fact that the main danger from plague, at present, arises solely from endemic foci which the pandemic in its march over the country has set up here and there. In these areas the disease smoulders among rodents, and now and then it flares up in epizootics of large proportions which in turn lead to severe epidemics, because the housing conditions in the rural areas are no better to-day than they were 45 years ago, when the wave of the present pandemic struck India. It is reasonable to assume from the past records of plague in the Peninsular India that some such endemic foci exist in the Deccan Plateau and they will always remain potent sources of danger. Because of them we cannot depend on the disease disappearing entirely through natural causes, as it has done in

countries where high standard of living and housing has broken the vicious connection between rat and man. Here active measures based on a definite understanding of these endemic foci must be adopted.

For an effective control of this disease we must gain an adequate knowledge of factors which favour endemicity. The first step in this direction should be a study of factors which exist in the area where plague persists. A knowledge of these factors would give us an insight into conditions that produce endemic foci and thereby enable us to map out all endemic foci in the Deccan Plateau and elsewhere in India. The detection of these endemic foci which are definitely increasing at the present time will enable us to exterminate them at a reasonable cost, and if that is not possible, we can at least maintain a strict surveillance over them in order to protect the adjoining areas.

In order to prosecute these studies on proper lines, it is necessary to survey those areas that have suffered from plague during the past five years and to find out the state of infection both in domestic and wild rodents. With this object in view, Barsi town with its surrounding infected villages was selected and a well equipped laboratory was opened there from July 1940 to September 1941. After the disappearance of plague in the Barsi Taluka, the Plague Research Field Laboratory was shifted to Dharwar town in October 1941. Two touring units are stationed near the infected villages. The collections of wild and domestic rodents from these villages are daily examined at the Field Laboratory for plague infection. The emulsions of tissues and ectoparasites of wild and domestic rodents are inoculated daily into white mice with a view to detect infection in them, as it is a well-known fact that fleas and rodents may harbour infection in nature without showing any apparent signs of plague. The results of the investigations of the inquiry during the years 1940 and 1941 are given in the form of a summary in Part II of this report.

Several amples of rat-traps were received during the year for examination and expert advice from different local bodies and firms. Suggestions for improving their construction were given to them.

During the year several requests about the identification of fleas and other insects and general information about insects and their control were received, and they were complied with. Live specimens of *X. cheopis* were supplied to the Superintendent, D. Ward, Bombay Municipality and 500 spirit preserved specimens of the rat-flea were sold to the General Manager, Oriental Science Apparatus Workshop, Ambala Cantonment. A gift of an Elongated Improved Wonder Rat Trap was made to Dr. J. B. Grant, Director, All India Institute of Hygiene and Public Health, Calcutta for transmission to Dr. E. Landaner, Peiping Union Medical College.

Mr. D. M. Munshi, B.Sc., St. Xavier's College, has been working as a part time research student ever since December 1940. He is engaged in the study of Morphology of the proventricular valve of the rat-fleas of the genus *Xenopsylla*. Dr. M. Sharif visited the following places for the period noted against them in connection with the Plague Recrudescence Inquiry in Sholapur and adjoining districts :—

(1) Sholapur ..	.. 14th March, 1940.
(2) Belgaum ..	.. 5th to 7th June, 1940.
(3) Poona ..	.. 8th to 13th June, 1940.
(4) Barsi ..	.. 20th July to 25th September; 11th to 25th October; 4th to 20th November; 3rd to 21st December, 1940; 4th to 27th January; 11th to 26th February; 11th to 26th March; 1st to 8th April; 28th April to 16th May; 9th to 20th June; 11th to 24th July; 12th to 22nd August; 14th to 20th September, 1941.
(5) Dharwar ..	.. 8th to 13th September; 8th to 28th October; 13th to 26th November; 11th to 23rd December, 1941.

A. *Examination of Bombay City Rats*

The examination of domestic rats and other rodents of the Bombay City and Port Trust Docks for evidence of plague infection was continued during the two years.

A permanent establishment is maintained by the Municipality of Bombay for catching rats, and about 2,000 rats are caught every night in the city. These are received in the Institute with locality records on labels tied to their tails. Out of these only dead rats are dissected and examined for post-mortem signs of plague. In doubtful cases smears and cultures are made. Besides providing valuable information on the subject of epidemiology, this work is intended to give the municipal authorities the first signal of any plague epizootic in a part of the city with a view to detect and control plague in the city.

In this report correct zoological nomenclature of the Bombay City rodents is adopted. The specific names given in previous reports were based mostly on the identifications of the members of the Indian Plague Commission, most of which were unfortunately wrong. In order to

avoid confusion, the correct identifications of these rodents along with previous specific names are given below :—

1.	*Rattus rattus rufescens*	=	*Rattus rattus*
2.	*Rattus norvegicus*	=	*Rattus norvegicus*
3.	*Gunomys kok*	=	*Gunomys varius*
4.	*Bandicota malabarica*	=	*Bandicota indica*
5.	*Mus dubius*	=	*Mus musculus*
6.	*Suncus cæruleus*	=	*Crocidura caerulea*

Tables giving the monthly totals of rats received and the number out of them that were examined are given below. During the two years no plague infected rat was detected and no human plague case occurred in the City and the Bombay Port Trust Docks.

TABLE I

Rats received at the Institute from the Bombay Municipality during 1940 and 1941

Months	1940			1941		
	Alive	Dead	Total	Alive	Dead	Total
January ..	38,331	31,111	69,442	26,876	28,152	55,028
February ..	33,684	27,545	61,229	22,824	24,938	47,762
March ..	32,833	30,221	63,054	23,594	27,021	50,615
April ..	34,792	28,475	63,267	22,693	25,870	48,563
May ..	29,017	25,053	54,070	19,501	23,825	43,326
June ..	26,946	25,980	52,926	15,186	21,871	37,057
July ..	26 264	24,980	51,244	21,993	25,921	47,914
August ..	29,362	24,288	53,650	33,572	29,022	62,594
September ..	26,312	23,344	49,656	30,367	34,329	64,696
October ..	32,369	29,170	61,539	27,827	35,445	63,272
November ..	28,565	25,602	54,167	28,561	36,491	65,052
December ..	27,021	29,958	56,979	29,937	39,829	69,766
Total ..	365,496	325,727	691,223	302,931	352,714	655,645

TABLE II

Examination of dead rats for plague during 1940 and 1941

Months	Dead rats examined in 1940	Dead rats examined in 1941	Found plague infected in 1940 and 1941	
			Rattus norvegicus	Rattus rattus rufescens
January	21,246	17,871		
February	19,692	16,420		
March	17,865	16,445		
April	18,772	16,370		
May	18,201	17,491		
June	15,621	14,415	*Nil*	*Nil*
July	17,246	18,394		
August	15,135	14,867		
September	13,573	16,805		
October	18,086	18,869		
November	17,845	21,448		
December	15,322	17,506		
Total ..	208,604	206,901		

TABLE

Showing the number and percentage of different species of rats.

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Rattus rattus rufescens.	7,674	24·3	6,867	24·3	5,931	26·4	6,461	22·9	5,467	21·2	3,348	15·1
Rattus norvegicus ..	7,454	23·6	6,541	23·1	4,931	21·9	6,917	24·5	6,579	25·6	4,897	22·1
Gunomys kok ..	11,403	36·1	10,159	35·9	7,822	34·8	10,262	36·3	8,969	34·8	10,149	45·8
Bandicota malabarica ..	478	1·5	434	1·5	304	1·4	291	1·0	206	0·8	186	0·8
Mus dubius ..	1,392	4·4	1,421	5·0	1,050	4·7	1,155	4·1	1,173	4·6	935	4·2
Suncus cæruleus ..	2,314	7·3	2,082	7·4	1,677	7·4	2,328	8·2	2,263	8·8	1,812	8·2
*Unidentifiable ..	890	2·8	773	2·8	781	3·4	859	3·0	1,085	4·2	854	3·8
†Total ..	31,605	100·0	28,277	100·0	22,496	100·0	28,273	100·0	25,742	100·0	22,181	100·0

*Rats included under this head though reported to have been caught

†These totals do not agree with the totals of live rats in Table I, because

III

trapped alive by Municipal Corporation, Bombay, during 1940

July		August		September		October		November		December		For whole period	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
4,711	20·5	5,760	23·4	5,054	25·3	9,479	38·4	7,338	29·8	4,617	26·3	72,707	24·9
5,545	24·1	6,074	24·6	5,050	25·3	5,390	21·8	5,888	23·9	4,549	26·0	69,815	23·9
8,653	37·6	9,105	36·9	6,615	33·2	6,184	25·0	7,648	31·0	5,697	32·5	102,366	34·8
202	0·9	162	0·6	145	0·7	144	0·6	180	0·7	195	1·1	2,927	1·0
955	4·1	882	3·7	917	4·6	948	3·8	934	3·8	833	4·8	12,595	4·3
2,121	9·2	1,934	7·8	1,405	7·0	1,707	6·9	1,912	7·7	1,260	7·2	22,815	7·9
829	3·6	739	3·0	776	3·9	839	3·5	752	3·1	367	2·1	9,544	3·2
23,016	100·0	24,656	100·0	19,962	100·0	24,691	100·0	24,652	100·0	17,518	100·0	293,069	100·0

alive, were received at the Institute dead often mutilated and even putrid.
rats received on Sundays and holidays were not classified.

TABLE

Showing the number and percentage of different species of rats

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Rattus rattus rufescens.	5,827	28·2	4,131	23·4	4,113	24·0	3,875	22·6	3,577	21·5	3,003	24·2
Rattus norvegicus ..	4,524	21·9	4,202	23·8	4,139	24·2	3,890	22·6	3,398	20·5	2,456	19·8
Gunomys kok ..	7,114	34·5	5,564	37·2	5,824	34·0	6,741	39·2	7,053	42·4	4,892	39·5
Bandicota malabarica.	146	0·7	130	0·7	105	0·6	139	0·8	138*	0·8	136	1·1
Mus dubius ..	1,012	4·9	709	4·0	898	5·3	742	4·3	740	4·5	516	4·2
Suncus cærulcus ..	1,382	6·7	1,317	7·5	1,446	8·4	1,299	7·5	1,085	6·5	944	7·6
*Unidentifiable ..	634	3·1	599	3·4	604	3·5	516	3·0	630	3·8	449	3·6
†Total ..	20,639	100·0	17,652	100·0	17,129	100·0	17,202	100·0	16,621	100·0	12,396	100·0

* Rats included under this head though reported to have been caught alive

† These totals do not agree with the totals of live rats in Table I, because

IV

trapped alive by Municipal Corporation, Bombay, during 1941

July		August		September		October		November		December		For whole period	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
4,314	21·8	4,883	18·1	4,964	21·4	7,387	33·1	6,394	26·3	5,315	26·4	57,733	24·2
4,282	21·6	7,235	27·1	5,962	25·6	4,614	20·7	5,569	23·0	4,615	23·0	54,886	23·0
8,673	43·7	10,121	37·8	8,431	36·3	6,777	30·4	8,675	35·8	7,554	37·6	88,419	37·1
147	0·7	147	0·5	159	0·7	173	0·8	241	1·0	235	1·2	1,396	0·8
650	3·3	748	2·8	912	3·9	1,004	4·5	1,149	4·7	838	4·1	9,918	4·2
1,680	8·5	3,170	12·9	2,700	11·6	2,311	10·4	2,202	9·1	1,550	7·7	21,086	8·9
70	0·4	487	1·8	123	0·5	27	0·1	22	0·1	..	..	4,161	1·8
19,816	100·0	26,741	100·0	23,251	100·0	22,293	100·0	24,252	100·0	20,107	100·0	238,099	100·0

were received at the Institute dead, often mutilated and even putrid.
rats received on Sundays and holidays were not classified.

TABLE

Showing the number and percentage of different species

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Rattus rattus rufescens	2,766	11·3	2,178	9·6	2,047	10·0	2,187	9·9	2,204	10·2	1,781	9·2
Rattus norvegicus ..	8,161	33·4	7,380	32·4	7,046	34·3	7,038	31·8	7,035	32·7	5,561	28·8
Gunomys kok ..	8,120	33·2	8,204	36·0	6,956	33·9	7,901	35·7	7,330	34·0	6,888	35·7
Bandicota malabarica.	233	1·0	211	0·9	179	0·9	135	0·6	100	0·5	108	0·6
Mus dubius ..	1,816	7·4	1,566	6·9	1,488	7·3	1,382	6·2	1,394	6·5	1,139	5·9
Suncus cæruleus ..	1,744	7·1	1,771	7·8	1,706	8·3	2,142	9·7	1,989	9·2	2,116	11·0
Unidentifiable ..	150	0·6	153	0·7	190	0·9	129	0·6	138	0·6	144	0·7
Putrid ..	1,455	6·0	1,313	5·7	906	4·4	1,229	5·5	1,366	6·3	1,560	8·1
*Total ..	24,445	100·0	22,776	100·0	20,518	100·0	22,143	100·0	21,556	100·0	19,297	100·0

*These totals do not agree with the totals of dead rats in table No. I, because

V

of rats found dead in the City of Bombay during 1940

July		August		September		October		November		December		For whole period	
Number	Percentage.	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
2,420	11·4	2,496	13·0	2,375	14·4	3,063	14·5	3,259	15·5	2,900	16·8	29,676	12·0
6,311	29·8	5,218	27·1	4,659	28·3	6,509	30·7	6,526	31·0	5,776	33·4	77,220	31·2
6,892	32·5	5,695	29·6	4,798	29·1	6,524	30·8	5,892	27·9	4,590	26·5	79,790	32·3
141	0·7	158	0·8	120	0·7	193	0·9	270	1·3	243	1·4	2,091	0·9
1,346	6·3	1,451	7·5	1,507	9·2	1,704	8·0	1,761	8·4	1,721	9·9	18,275	7·4
2,768	13·0	2,240	11·6	1,956	11·9	2,511	11·8	2,554	12·1	1,603	9·3	25,100	10·1
136	0·6	117	0·6	114	0·7	93	0·4	137	0·6	92	0·5	1,593	0·6
1,204	5·7	1,895	9·8	938	5·7	618	2·9	685	3·2	376	2·2	13,545	5·5
21,218	100·0	19,270	100·0	16,467	100·0	21,215	100·0	21,084	100·0	17,301	100·0	247,290	100·0

rats received on Sundays and holidays were not classified.

TABLE

Showing the number and percentage of different species

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Rattus rattus rufescens	3,082	15·3	2,492	13·6	2,569	13·8	2,635	14·3	2,699	13·7	2,264	13·1
Rattus norvegicus ..	5,856	29·2	5,579	30·5	5,453	29·4	5,242	28·4	5,365	27·2	4,645	26·7
Gunomys kok ..	5,896	29·3	6,004	32·9	5,914	31·8	6,045	32·7	7,300	37·0	5,741	33·1
Bandicota malabarica	342	1·7	251	1·4	150	0·8	121	0·7	186	0·9	215	1·2
Mus dubius ..	2,695	13·4	2,094	11·5	2,286	12·3	2,210	12·0	1,809	9·2	1,392	8·0
Suncus cæruleus ..	1,860	9·3	1,787	9·8	1,903	10·3	1,842	10·0	1,569	8·0	1,364	7·8
Unidentifiable ..	..	..	..	..	73	0·4	117	0·6	132	0·7	158	0·9
Putrid ..	366	1·8	56	0·3	226	1·2	247	1·3	655	3·3	1,598	9·2
*Total ..	20,097	100·0	18,263	100·0	18,574	100·0	18,459	100·0	19,715	100·0	17,377	100·0

*These totals do not agree with the totals of dead rats in table No. I, because

VI

of rats found dead in the City of Bombay during 1941

July		August		September		October		November		December		For whole period	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
2,973	13·4	3,008	14·9	3,645	14·9	3,879	14·5	4,522	15·2	3,643	15·1	37,411	14·4
6,234	27·0	4,579	22·7	5,156	21·0	5,834	21·8	6,463	21·7	5,500	22·8	65,906	25·4
6,917	31·2	5,491	27·2	6,057	24·7	6,957	26·1	7,495	25·2	6,332	26·2	76,149	29·3
244	1·1	136	0·7	131	0·5	155	0·6	276	0·9	208	0·9	2,415	0·9
1,856	8·4	1,524	7·6	1,612	6·6	1,844	6·9	2,498	8·4	1,676	6·9	23,496	9·0
2,368	10·7	3,199	15·9	3,181	13·0	2,822	10·6	3,332	11·2	2,000	8·3	27,227	10·5
170	0·8	129	0·6	204	0·8	200	0·7	194	0·7	147	0·6	1,524	0·6
1,426	7·4	2,098	10·4	4,523	18·5	5,011	18·8	4,953	16·7	4,628	19·2	25,787	9·9
22,188	100·0	20,164	100·0	24,509	100·0	26,702	100·0	29,733	100·0	24,184	100·0	259,915	100·0

rats received on Sundays and holidays were not classified.

B. *Examination of Rats caught from*

These rats were received along with those of the other wards of the Bombay given in previous tables. A separate record of these rats giving the number and Health Commissioner. The following tables give summaries of these records.

TABLE

Showing the number and percentage of different species of rats

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Rattus rattus rufescens.	345	32·4	256	24·8	345	43·1	263	36·3	434	44·9	343	38·9
Rattus norvegicus ..	62	5·8	89	8·6	63	7·9	75	10·4	106	11·0	56	5·8
Gunomys kok ..	217	20·3	299	29·0	163	20·4	134	18·5	121	12·5	167	19·4
Bandicota malabarica.	23	2·2	33	3·2	7	0·9	12	1·6	5	0·5	12	7·5
Mus dubius ..	339	31·8	265	25·7	183	22·8	197	27·2	269	27·8	182	21·8
Suncus caeruleus ..	79	7·4	89	8·7	39	4·9	33	4·6	32	3·3	57	7·0
Unidentifiable ..	1	0·1	..	..	..	..	10	1·4	..	..	1	0·1
Total ..	1,066	100·0	1,031	100·0	800	100·0	724	100·0	967	100·0	818	100·0

Rats received on Sundays and

the Bombay Port Trust Docks

Municipality and have been already accounted for in the monthly totals percentage of the different species is also maintained at the instance of the Public

VII

caught alive from the Bombay Port Trust area in 1940

July		August		September		October		November		December		For whole period	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
499	46·0	470	46·7	345	41·2	373	46·0	377	42·2	267	40·0	4,317	40·3
63	5·8	61	6·1	59	7·1	78	9·6	68	7·6	55	8·2	835	7·8
193	17·8	159	15·8	179	21·4	111	13·7	208	23·3	98	14·6	2,049	19·1
19	1·8	8	0·8	8	0·9	4	0·5	12	1·3	24	3·6	167	1·6
256	23·6	272	27·1	195	23·3	207	25·6	172	19·2	167	25·0	2,704	25·3
54	5·0	35	3·5	51	6·1	37	4·6	57	6·4	58	8·6	621	5·8
..	..	..	..	..	..	..	..	...	...	...	..	12	0·1
1,084	100·0	1,005	100·0	837	100·0	810	100·0	894	100·0	669	100·0	10,705	100·0

holidays were not classified.

TABLE

Showing the number and percentage of different species of rats

Species	January		February		March		April		May		June	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Rattus rattus rufescens.	472	53·2	276	42·1	1,269	43·2	277	36·0	324	35·7	298	40·1
Rattus norvegicus	57	6·4	35	5·3	31	5·0	79	10·3	69	7·6	61	8·2
Gunomys kok ..	90	10·1	138	21·0	109	17·5	160	20·8	205	22·6	157	21·1
Bandicota malabarica ..	7	0·8	6	0·9	7	1·1	7	0·9	6	0·7	13	1·8
Mus dubius ..	226	25·4	131	20·0	165	26·5	172	22·4	229	25·2	160	21·5
Suncus cœruleus ..	36	4·1	70	10·7	37	5·9	73	9·5	74	8·2	54	7·3
Unidentifiable ..	..	..	..	..	5	0·8	1	0·1	..	..	..	..
Total ..	888	100·0	656	100·0	623	100·0	769	100·0	907	100·0	743	100·0

Rats received on Sundays and

VIII

caught alive from the Bombay Port Trust area in 1941

July		August		September		October		November		December		For whole period	
Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
472	48·7	364	50·9	362	43·3	462	46·5	397	39·3	395	49·9	4,368	44·1
81	8·4	41	5·7	37	4·4	30	3·0	51	5·0	27	3·4	599	6·0
200	20·7	99	13·8	160	19·1	166	16·7	138	13·7	157	19·9	1,779	18·0
4	0·4	11	1·5	5	0·6	11	1·1	10	1·0	8	1·0	95	1·0
167	17·2	145	20·3	220	26·3	250	25·2	334	33·0	146	18·5	2,345	23·7
43	4·4	56	7·8	53	6·3	75	7·5	81	8·0	58	7·3	710	7·2
2	0·2	..	..	..	..	..	..	..	..	..	..	8	0·0
969	100·0	716	100·0	837	100·0	994	100·0	1,011	100·0	791	100·0	9,904	100

holidays were not classified.

C. *Examination of Rats from Other Sources.*

During the years 1940 and 1941, 154 and 593 dead rats and 39 and 58 smears from dead rats respectively were received from different towns of the Bombay Province. As a result of their examination 9 rats were reported upon as being plague infected in 1940 and 8 in 1941 from towns as shown in the following table.

TABLE IX

Showing the different localities in the Bombay Province from which plague infected rats were received during the years 1940 and 1941

Name of Town	Number of plague infected rats in 1940	Number of plague infected rats in 1941
Ahmednagar	1	..
Belgaum	2	..
Indi	3	..
Poona	1	3
Ratnagiri	1	2
Sirsi	1	..
Bhatkal	..	1
Malvan	..	2
Total ..	9	8

D. *Training of Health Personnel*

The following persons were given training in plague preventive measures for the period noted against their names :—

(1) Mr. S. M. Dave, M.B.B.S., B.Hy. (Bom.), B.M.S. (II) from 12th to 18th February 1940.

(2) Dr. V. N. Ambekar, M.D. (Bom.), B.M.S. (II) from 14th to 30th March 1940.

(3) Mr. P. L. Powar, M.B.B.S. (Bom.), B.M.S. (II) from 3rd to 16th May 1940.

(4) Mr. A. V. Dave, M.B.B.S., Medical Officer, Plague Recrudescence Inquiry, from 2nd to 19th July 1940.

(5) Mr. R. G. Nadkarni, L.C.P.S., Assistant Technician, Plague Recrudescence Inquiry, Barsi, from 23rd July to 6th August 1940.

(6) Six Laboratory Attendants, Plague Recrudescence Inquiry from 4th June to 19th July 1940.

(7) Mr. S. G. Puranik, L.C.P.S., Assistant Technician, Plague Recrudescence Inquiry, Barsi, from 28th July to 6th August 1941.

(8) Mr. M. Qutubuddin, M.Sc., Public Health Department, H. E. H. the Nizam's Government, Hyderabad-Deccan from 18th July to 10th October 1941 (also training in Medical Entomology).

(9) Mr. K. H. Tergaonkar, Clerk, Director of Public Health for the Government of Bombay, from 1st to 10th December 1941 (also training in cholera and plague inoculation technique).

(10) Mr. D. T. Visal, Medical Officer, Poona City Municipality from 8th November to 9th December 1941.

(11) The 1940 and 1941 classes of Sanitary Inspectors were given short-course of training in rat examination and rat and flea destruction.

The following persons were given training in media making for the period noted against their names :—

(1) Mr. L. B. Deo from 18th September to 24th October 1940.

(2) Mr. Shinde, Laboratory Assistant, Cama Hospital, from 1st to 29th November 1941.

SECTION IV

DIAGNOSTIC DEPARTMENT

A. *Bacteriological Section*

This section examined clinical material from the various hospitals in the City and the mofussil places in the presidency with a view to help in arriving at the correct diagnosis of the different diseases. During the years 1940 and 1941, 13,169 and 11,168 examinations respectively were carried out. The following table gives an idea of some of the important examinations carried out :—

Nature of examination	1940		1941	
	Number of samples examined	Number of positive samples	Number of samples examined	Number of positive samples
Wassermann Test	4,259	1,201	3,796	1,159
Kahn Test	4,226	1,194	3,724	1,065
Blood smears for Malaria	113	19	61	3
Sputum and urine for Tuberculosis by smears and animal inoculation.	148	21	74	23
C. S. F. smears and cultures for Meningitis	289	32	149	12
Throat swab-smears, etc. for Diphtheria.	120	26	59	13
Nasal smears for Leprosy ..	99	85	142	105
Friedman's test for pregnancy ..	39	13	80	30

Besides this a number of autovaccines were prepared and supplied as also diagnostic group sera and various media.

B. *Pathological Section*

Of the 1,203 pathological tissues examined during the period under review, 116 were diagnosed as benign tumours and 147 as malignant tumours. Of the malignant tumours 68·0 per cent. were carcinomas, of which 70·0 per cent. originated in the female reproductive organs and the breast. Of the four rodent ulcers diagnosed, three were from rather uncommon sites, e.g. scapula, back and buttocks.

Besides the above specimens, 266 brains from suspected rabid dogs were examined for the presence of Negri bodies and 175 were found to be positive.

C. *Section of Public Health Chemistry and Bacteriology*

This section mainly examined samples of waters for their fitness for drinking and industrial purposes, samples of ghee and butter for detection of adulteration and sausages and other foodstuffs for their fitness for human consumption. Besides a number of samples of insecticidal sprays were tested for their toxicity against mosquitoes. The following table gives a summary of the various examinations carried out :—

Sample examined	Nature of examination done	Number of samples examined
Water	Fitness for drinking purposes	56
Water	Fitness for swimming purposes ..	27
Water	Fitness for industrial use	4
Milk	Adulteration	23
Ghee and butter ..	Do.	16
Sausages	Fitness for human consumption ..	34
Beer	For presence of live yeast	4
Septic tank effluents ..	For the degree of purification	3
Insecticidal sprays ..	For toxicity against mosquitoes ..	7

SECTION V

Department of Pharmacology

The department is still carrying on its work under the auspices of the Indian Research Fund Association. Dr. B. K. Nandi who was appointed by the Association as a Chemist, resigned his appointment at the end of 1940 to take up a post elsewhere. Capt. Inderjit Singh who was working

in the department as an honorary worker also left the Institute in the beginning of the year. Dr. H. S. Mahal was appointed in Dr. Nandi's place in 1941. Mr. B. V. Patel and Mr. R. N. Kulkarni are working in the department as research scholars.

Research work in the department mainly dealt with the problems of malaria. The action of Sulphathiazole in monkey malaria was investigated and it was found that a single dose of 0·5 g. was able to bring about a complete disappearance of parasites from the peripheral blood in rhesus monkeys infected with *Plasmodium knowlesi*. Of the new sulphanilamides prepared by the Chemotherapy Department, 2 N^1-sulphanilamido-5-ethylthiazole was shown to possess as good an anti-malarial activity in monkey malaria as sulphathiazole. Whether these drugs would exert a similar activity against human strains of malaria is a point still under investigation.

The problem of immunity in monkey malaria was pursued further during the two years under report and sufficient evidence has been collected to show that both the humoral and cellular factors are concerned in the immunity. It was also shown that the cellular factors exerted their influence more directly while it was suggested that the part played by humoral factors was probably an indirect one.

During the year 1941 investigations were carried out to determine the best form of local treatment for burns and it was found that sulphathiazole-cod liver oil combination was a very satisfactory agent for local treatment of burns. Similarly sulphathiazole was found to be a very useful remedy in experimental crushed and infected wounds.

Another interesting investigation was carried out in connection with the problem of 'Sleep' and the results were incorporated in the Presidential address delivered by Dr. B. B. Dikshit before the Physiology Section of the Indian Science Congress held at Benares early in 1941. Chemical agents responsible for natural sleep were investigated and a case made in favour of acetylcholine which deserves a careful consideration as a "Sleep-producing hormone."

Biological Assay of Drugs.—As usual samples of drugs were sent to the department for biological assays. As pointed out last year, the department being mainly a research department run by the Indian Research Fund Association cannot undertake this work to any important extent. Similarly quite a large number of drugs are sent to the department for investigation of their therapeutic properties but this kind of work also can be undertaken to a very limited extent. No drugs are accepted for investigation of their properties unless they are sent by a qualified medical practitioner. During the two years under report several examinations of pharmacopial drugs were made on behalf of some commercial firms which sent their products for biological assays.

Besides the research work various items of miscellaneous investigations were undertaken in the department. One such item was an interesting medico-legal investigation concerning a murder case from Indore. It

was alleged that the murderer had injected cobra venom into the victim and authorities wanted to verify that an empty syringe and an empty bottle found in possession of the accused contained or had sometime contained cobra venom. By using some of the modern physiological tests it was possible to prove that both the syringe and the bottle contained cobra venom, and this conclusion was confirmed by applying some specific tests such as protection given to mice by the specific antivenene.

SECTION VI

Department of Chemotherapy

During the years under review about 50 pounds of sulphathiazole were prepared for experimental studies and for the clinical trials in plague referred to in Part II of the report. In the course of this preparation the optimum conditions for the large scale production of this drug have been studied and it appears possible to produce this drug at a cost much lower than that prevailing in the market. About 10 pounds of sulphaguanidine was also prepared for experimental studies. The production of 2-Methyl-1 : 4-naphthoquinone, the synthetic substitute for vitamin K now in use, has been studied.

Synthesis of new derivatives of sulphanilamide was continued with a view to obtain compounds better than sulphathiazole and also to study systematically the relationship existing between antibacterial action and chemical constitution. Derivatives of sulphanilamide with various carbo and heterocyclic substituents at the sulphonamide radical have been synthesised and studied. Many compounds related to sulphathiazole with additional alkyl or substituted alkyl substituents at the positions 4 and /or 5 of the thiazole ring and the sulphonamide radical have also been synthesised and studied. From these studies some interesting relationship existing between chemical constitution and chemotherapeutic action has emerged. Of the compounds studied, 5-ethyl sulphathiazole, 5-isopropyl sulphathiazole, N^1-methyl sulphathiazole, sulphathiazoline and 4-methyl sulphapyrimidine showed good therapeutic activity and are being subjected to more detailed study in the Institute.

SECTION VII

Department of Biochemistry

Medical practitioners and hospitals are making increasing use of the Biochemical Unit for the various bio-chemical examinations required by them in the diagnosis of disease. The Unit renders great help also to the other departments of the Institute in their research work.

1. *Nutritional Work.*—As the biological activity of vitamin D content of an unknown substance depends on the dose given, experimental work has been undertaken to establish a dosage response relation for the unknown and the standard for the assay of vitamin D by the percentage ash content of bone test in rats reared on the stock colony diet used in this Institute.

2. *Energy Metabolism.*—The correlation between urinary nitrogen which can be taken as an index of protein metabolism and basal metabolism is being studied with a view to determine whether the higher basal metabolism in certain individuals is due to higher protein metabolism.

3. *Blood Urea Clearance.*—The study of blood urea clearance of normal Indians was continued. More normal Indian subjects were examined and the individual variation in the blood urea clearance values in some subjects has been studied during the period under report. The study has been completed and results published.

4. *Normal Urine Constituents.*—Study of chemical composition of 24-hour collections of urine of normal Indians was undertaken. Urines of one hundred and seventeen normal Indian subjects have been examined. The results will soon be sent for publication.

5. *Broth Experiments.*—Experimental work with a view to improve the meat digest media used in the manufacture of the Plague Vaccine in the Institute has been undertaken.

6. *Serum Concentration.*—During the period under report, 20·2 litres of antiplague serum were concentrated according to the method already described (Rep. of the Haffk. Inst. 1932-35, p. 26). 9270 cc. of anti-typhoid serum also were concentrated.

7. *Routine Examinations.*—In the department, 2,597 routine examinations were carried out; bloods 991, urines 587, cerebrospinal fluids 743, faeces 32, milk 40, ascitic fluids 2, and 202 special tests such as 78 blood urea clearance, 66 gastric analyses, 39 basal metabolic rate determinations and 19 glucose tolerance tests. Most of the routine examinations were determinations of non-protein nitrogenous constituents such as urea, uric acid and creatinine, sugar, serum calcium, haemoglobin, Van den Bergh reaction, and serum proteins.

SECTION VIII

Anti-Rabic Department

During the years under review, 6,715 persons attended for anti-rabic treatment at the Institute. Out of these 6,001 completed the full course of treatment, 698 were advice cases and 16 discontinued treatment of their own accord. These advice cases were given two injections each and advised to keep the offending dog under observation. And as the dogs were still healthy at the end of 14 days' period of observation, no further treatment was given. A period of 10 days used to be the period recommended for observation for offending dogs before. However, to be on the safe side, a 14 days' period of observation is now being recommended in view of the results obtained by Babes, Zaicharia and Pamponkis who reported "that dogs transmitted rabies by biting 14 days before the onset of symptoms" (Rosenau).

During the period under report the new classification of bites for purposes of treatment was introduced. The classification was on the lines agreed upon at the meeting of Pasteur Institute workers in Simla in 1939. The new classification is simple in that it grades the degree of bites as mild, moderate and severe, and the treatment is graded correspondingly.

During 1940 only one patient died of hydrophobia. He was a boy under 12 who was badly bitten on his face, arm and leg by a rabid dog in Calcutta. He received 5 injections of 1 per cent anti-rabic vaccine in Calcutta and was subsequently brought to Bombay. He was brought to the Institute and Class III treatment was started at once. This was, however, 10 days after the bite. The boy developed hydrophobia 6 days after treatment was started here and died 2 days later. No case of hydrophobia was reported in 1941.

A total of 15,201 anti-rabic treatments was issued to the centres. Of these 875 were issued for veterinary use.

The following three new anti-rabic centres were opened during the period under report bringing the total of our centres to 103.

(1) Mangrol (Kathiawar) under the Chief Medical Officer.

(2) Yeotmal (Berar) under the Civil Surgeon.

(3) Jawhar State under the Chief Medical Officer.

The details of treatment carried out at the Institute and at out centres in 1939, 1940 and 1941 are summarised below :—

Details	1939	1940	1941
Number of persons applied for the treatment ..	3,292	3,311	3,404
Advice cases	326	390	308
Fully treated	2,960	2,916	3,085
Percentage of health returns	97·8	98·1	Awaited.
Cases of hydrophobia	2	1	Nil
Mortality rate	0·06	0·03	Nil
Number of anti-rabic centres	101	102	103
Number of treatments sent to the centres ..	6,876	6,939	7,387
Number of treatments sent to the Veterinary Surgeons.	350	278	597

Table showing the number of brains received and examined at the Institute for evidence of rabies during the years 1940 and 1941

Details	1940		1941	
	Number	Percentage	Number	Percentage
Positive for rabies (Negri bodies present) ..	93	76·2	82	56·9
Negative for rabies (no Negri bodies present)	27	22·1	57	39·6
Received in a state unfit for examination	2	1·7	5	3·5

Source of brains received and examined for rabies :—

	1940					1941				
	Positive	Negative	Unfit for examination	Total	Percentage	Positive	Negative	Unfit for examination	Total	Percentage
Bombay City and Suburbs.	39	12	0	51	41·8	30	24	0	54	37·5
Outstations ..	54	12	2	71	58·2	52	33	5	90	62·5
Total ..	93	24	2	122	..	82	57	5	144	..

Statement showing the classification of different animals with the results of their brain examination for rabies.

Animal	1940				1941			
	Positive	Negative	Unfit for examination	Total	Positive	Negative	Unfit for examination	Total
Dog	88	22	1	111	79	52	4	135
Buffalo	..	..	..	..	2	1	0	3
Bullock	0	0	1	1	..	..	..	..
Cat	1	0	0	1	0	0	1	1
Cow	1	0	0	1	..	..	..	..
Donkey	0	1	0	1	..	..	..	..
Goat	1	0	0	1	..	..	..	..
Horse	0	2	0	2	0	1	0	1
Human	0	1	0	1	..	..	..	..
Jackal	1	0	0	1	0	1	0	1
Mule	..	..	..	..	1	0	0	1
Pig	0	1	0	1	..	..	..	..
Rabbit	..	..	..	..	0	1	0	1
Tiger	..	..	..	..	0	1	0	1
Wolf	1	0	0	1	..	..	..	..
Total ..	93	27	2	122	82	57	5	144

SECTION IX

Income and Expenditure

The following table shows the income and expenditure of the Haffkine Institute :—

Receipts		Expenditure	
1939–40			
	Rs.		Rs.
XXVIII-Public Health— Sale proceeds of sera, vaccines and other materials.	318,216	39-D-Public Health— D-Bacteriological Laboratories, Haffkine Institute, and 39-E-Public Health— E-Pasteur Institute—	
		Pay of Officers ..	92,497
		Pay of Establishment ..	87,689
		Allowance, honoraria, etc.	7,959
		Contingencies ..	151,159
Total Receipts ..	318,216	Total Expenditure ..	339,304
1940–41			
	Rs.		Rs.
XXVIII-Public Health— Sale proceeds of sera, vaccines and other materials.	486,118	39-D-Public Health— D-Bacteriological Laboratories, Haffkine Institute, and 39-E-Public Health— E-Pasteur Institute—	
		Pay of Officers ..	90 010
		Pay of Establishment ..	94,185
		Allowances, honoraria, etc.	8,334
		Contingencies ..	210,780
Total Receipts ..	486,118	Total Expenditure ..	403,309

PART II

RESEARCH WORK

I. Antiplague Serum, Sulphathiazole and Sulphapyridine in the Treatment of Bubonic Plague

(SOKHEY AND WAGLE)

During the two years under review three opportunities presented themselves to test in the field the comparative curative value of Haffkine Institute antiplague serum, sulphathiazole, sulphapyridine and sulphathiazole cum antiplague serum in the treatment of bubonic plague. An attempt was also made to work out optimal dosages of the sulphonamides used.

The first field trial was conducted at Bettiah, Bihar, from January 1, 1940 to April 15, 1940. An epidemic had broken out at Bettiah. The Bettiah Raj and the Director of Public Health, Bihar, invited the Institute to send a research unit. The Bettiah Raj erected a temporary plague hospital and provided other facilities for a controlled trial to be conducted. A research unit with the necessary laboratory equipment was sent from the Haffkine Institute. The cost of this research unit was borne by the Indian Research Fund Association during this trial as well as during the subsequent field trials detailed below. In all 294 cases were treated, out of which only 275 proved to be cases of plague. This trial was utilised to determine the relative value of antiplague serum (Haffkine Institute), sulphapyridine and sulphathiazole in the treatment of bubonic plague.

When the very successful results obtained at Bettiah became known, the late Sir Akbar Hydari, President of the Council of State, and Nawab Khusru Jung Bahadur, Member in charge of Medical Department of H. E. H. the Nizam's Dominions, showed great interest as plague is endemic in some parts of the State. They invited the Institute to utilise the facilities which the State was willing to provide to conduct further studies. Dr. Hyder Aali Khan, Director, Medical and Public Health Departments, deputed Dr. M. Farooq, Assistant Director, to visit the Institute to work out details. By mutual consultation, Latur, a town with a population of about 30,000 situated in the midst of the endemic area, was selected for a field trial. The State erected a temporary hospital and provided other facilities. A unit from the Institute went to Latur and conducted the second field trial from November 1, 1940 to April 15, 1941. At Bettiah we had used, we believed, the sulphonamides in too small a dosage and were not equipped to control the dosage by measuring the concentration of the drug in blood. We, therefore, utilised the second trial to give sulphathiazole and sulphapyridine in larger dosage and controlled the dose by estimating the concentration of the drug in blood 4 hours, 8 hours, and 24 hours after the administration of the drug and once daily thereafter. During this field trial 312 cases were admitted to hospital. To encourage

patients to come to the hospital even cases with fever which were obviously not cases of plague were admitted, thus only 267 cases proved to be cases of plague. It is to be noted that very few cases occurred at Latur itself. Most of the cases came from villages within a radius of about 30 miles. Patients were brought to Latur in a motor van and bullock carts over rough dirty tracks. Consequently most of the cases arrived at the Hospital with the disease well advanced and made still worse by jolting over rough roads. This has important bearing on the results to be given below.

For the third field trial the Institute was again invited to Latur and worked there from December 1, 1941 to March 31, 1942. The hospital erected for the previous trial was again utilised and again very few cases came from Latur itself, as the disease was limited to the surrounding villages as on the previous occasion. This time 153 cases were admitted and out of these only 140 proved to be cases of plague. This trial was utilised to compare the curative value of sulphathiazole along with that of sulphathiazole cum antiplague serum.

Methods

Diagnosis.—On admission, before any treatment was given, 0·5 cc. of blood was drawn from a vein and planted in equal quantities on two agar slopes. This was done to determine the presence or absence of plague septicæmia at the time of admission and its severity if present. These slopes were incubated at room temperature for two days and if the cultures remained sterile bubo was punctured with a syringe and the fluid drawn up planted on agar slopes to confirm diagnosis. If 0·25 cc. of blood gave less than 10 colonies, it was noted as a case of mild septicæmia while cases with over 10 colonies in 0·25 cc. of blood were considered cases of severe septicæmia. Many severe septicæmic cases showed over 1,000 colonies for 0·25 cc. of blood and some gave even a confluent growth.

During the field trials it was noticed that the most important single factor which decided the issue in human bubonic plague was the development and degree of septicæmia. If the lymph-gland prevented the spread of infection to the blood stream and the infection remained localised spontaneous recovery usually resulted. On the other hand if the organisms passed the lymph-gland and septicæmia resulted death almost invariably followed unless an effective curative agent was given to control the infection. Among the control cases, several, which had no septicæmia at the time of admission, developed septicæmia later and died. While with the three therapeutic agents under test septicæmia seldom developed if it was not present at the time of the exhibition of the agent. Thus a truer picture of the results of a given treatment is obtained if only those cases are considered which had septicæmia at the time the treatment was started. For this reason results of each trial are given in two tables. One table shows the results of the treatment in " all cases ", and the other of " cases with plague septicæmia at the commencement of the treatment ".

Measurement of the Virulence of Strains.—We have measured the virulence of hundreds of strains of the plague organism obtained during these trials from the blood and the buboes. We were surprised to find that every strain obtained from a human case of plague with or without septicæmia proved to be highly virulent, i.e., 5 organisms (even less) produced almost 100 per cent mortality in mice,—Haffkine Institute-inbred strain. This observation is of some interest because Girard and Robic claim to have isolated a strain from a human case which they found to be avirulent.

Division of Cases.—Different treatments were given in strict rotation, no selection of cases was made. In spite of this fact the number of cases given in the tables below do not even up for different treatments. There were several reasons for this. It took not less than 48 hours to make a definite diagnosis bacteriologically. Thus some patients after the commencement of treatment proved not to be cases of plague. Further as stated above patients came from long distance over rough roads in springless bullock carts and arrived at the hospital in such advanced state of the disease that they died within a few hours after the treatment was commenced. Cases moribund at the time of admission and dying within 24 hours of admission had to be kept out of account as we found that the sulphonamides were not effective within such a short period. In the first trial detailed here, on the basis of our experience of treatment of cases of plague with antiplague serum, only cases dying within 12 hours of admission were excluded from the survey of results given in the 1939 report. But in the subsequent two trials detailed here, when we were in a position to measure the concentration in blood of the sulphonamides used, we found that the therapeutic concentration of the drug was not reached till 6 to 8 hours after the ingestion of the drug. This called for the exclusion from reckoning of cases dying within 24 hours. Then again a small number of cases left hospital against advice while still febrile. We have also decided to deal with separately the very small number of cases of pneumonic plague admitted during these three trials under review—10 in all.

Concentration of Sulphonamides in Blood.—During the first trial of the present series, at Bettiah, we were not equipped to measure the concentration of sulphapyridine and sulphathiazole in blood. We, therefore, empirically adopted the following dosage for each drug, 3·5 g. the first day and 3 g. during each succeeding day for 7 days. Of course the drug was stopped earlier if the patient fully recovered and in some cases continued it longer than 7 days. We were thus not sure whether we had used an adequate dosage. During the next two trials at Latur we controlled the dosage of the sulphonamide by measuring the concentration of the drug in blood at 4 hours, 6 hours, 8 hours and 24 hours during the first day and thereafter once daily. In the case of sulphathiazole we first attempted to get a concentration of 10–20 mg. per cent. This necessitated a dosage of 14 g. the first day and 9 g. each succeeding day. We tried this dosage in 56 cases but found it rather toxic. We then reverted to a dosage of 10 g. the first day and 7·5 g.

per day the next 4 to 5 days and a smaller dose later. This gave a concentration of 5–10 mg. per cent. With the smaller dose the improvement of the condition was as good as with bigger dose. Unfortunately we could not try again at Latur the still smaller dose used at Bettiah. Thus the question of optimal dosage in the case of sulphathiazole remains undecided, but we can say this much that 7 to 10 g. of the drug can be given safely without any serious toxic symptoms. In the case of sulphapyridine at Latur we tried a dose of 9·5 g. the first day and 8 g. per day for the next 4 days and a smaller dose later when necessary. This dosage produced very serious toxic symptoms and we reduced the dosage to 8 g. the first day and 3-4 g. per day during the next 4 days. The smaller dose gave a concentration in blood of 5–10 mg. per cent.

Controls.—During the field trial at Bettiah, the first trial of the present series, like the field trials reported in the previous reports, alternate admissions were treated with iodine intravenously. This treatment was in vogue in the hospitals at that time. The cases so treated were considered as controls. But during the succeeding two trials we did not keep any controls, because during the first trial of this series and the previous field trials reported in the Annual Reports for 1936 and 1938, we had had 149 controls and these had provided all the necessary information. Control cases which had septicæmia at the time of admission with 10 colonies or over had a mortality of 96 to 100 per cent and taking all septicæmia together the death rate was higher than 90 per cent. Though it is true that a number of controls which had no septicæmia at the time of admission developed it later and died of the disease, still we have relied for forming a judgment of the value of the three agents under test on the cases which had septicæmia at the start of the treatment.

Results

*First Field Trial—Bettiah**

(January 1, 1940 to April 15, 1940)

Total cases of plague treated		275
Excluded from reckoning—		
Cases moribund at the time of admission and died within 24 hours of admission.	18	
Unclassified into septicæmic and non-septicæmic cases.	11	
Cases treated with sulphapyridine cum serum.	6	
Pneumonic plague	6	
Cases left hospital while still febrile ..	4	
		45
Cases accounted for		230

*A summary of these results was given in the Annual Report for 1939; at that time we had excluded cases which had died within 12 hours of admission; but in this table we are excluding cases which died within 24 hours for reasons given above.

Treatment and Dosage.

Antiplague serum .. 40 cc. a day for 3 to 5 days.

Sulphapyridine .. 3·5 g. first day and 3 g. per day for about 7 days.

Sulphathiazole .. Do.

Experiment was commenced by giving antiplague serum to the first case, iodine solution intravenously to the second case and sulphapyridine to the third case. This order was maintained till case No. 210 was reached when sulphapyridine was replaced by sulphathiazole.

All cases

Treatment	Number of cases	Deaths	Case mortality per cent
Antiplague serum	67	17	25·3
Sulphapyridine	52	12	23·0
Sulphathiazole	32	5	15·6
Iodine intravenously (Controls)	79	40	50·6
Total ..	230	74	..

Cases with plague septicæmia at the commencement of treatment

Treatment	Number of cases	Deaths	Case mortality per cent
Antiplague serum	30	17	56·6
Sulphapyridine	29	12	41·3
Sulphathiazole	12	5	41·6
Iodine intravenously (Controls)	37	35	94·5
Total ..	108	69	..

Second Field Trial—Latur

(November 1, 1940 to April 15, 1941)

Total cases of Plague treated			267
Excluded from reckoning—			
Cases moribund at the time of admission and died within 24 hours of admission		35	
Unclassified		2	
Treated with sulphanilamide		2	
Left hospital while febrile		7	
Combined treatment—sulphathiazole and serum		2	
Pneumonic plague		2	
			50
			217

Treatment and Dosage

Sulphathiazole.—First 56 cases received 14 g. the first day and 9 g. a day for the following 3 or 4 days and less thereafter, concentration of the drug in blood more than 10 mg. per cent. The remaining cases (91) received 10 g. on the first day and 7·5 g. a day for the next 3 or 4 days with reduced dose later : concentration of the drug in blood 5—10 mg. per cent.

Sulphapyridine.—The first 4 cases received 9·5 g. the first day and 8 g. per day for the next 3 or 4 days. This gave a concentration in blood of over 10 mg. per cent. All 4 cases developed severe toxic symptoms. Remaining cases received 8 g. the first day and 3 to 4 g. a day for the next 3 to 4 days and reduced dosage later if necessary: concentration of the drug in blood 5—10 mg. per cent.

The first 80 cases were treated with sulphathiazole and after that sulphathiazole and sulphapyridine were given in rotation to alternate admissions.

All cases

Treatment	Number of cases	Deaths	Case mortality per cent
Sulphathiazole	147	33	22·4
Sulphapyridine	70	21	30·0
Total ..	217	51	..

Cases with plague septicæmia at the commencement of the treatment

Treatment	Number of cases	Deaths	Case mortality per cent
Sulphathiazole	62	26	41·9
Sulphapyridine	33	19	57·5
Total ..	95	45	..

Third Field Trial—Latur

(December 1, 1941 to March 31, 1942)

Total cases of Plague treated			140
Excluded from reckoning—			
Moribund at the time of admission	..	25	
Leaving hospital against advice	..	2	
Pneumonic plague ..	..	2	
		—	29
			111

Treatment and Dosage

Admissions were treated in rotation with sulphathiazole cum antiplague serum and sulphathiazole alone. Sulphathiazole was given in dosage of 10 g. the first day and 7·5 g. a day for the subsequent 3 or 4 days with reduced dosage later. In combined treatment the dosage of sulphathiazole was kept the same and 40 cc. serum a day for 4 or 5 days was given in addition.

All cases

Treatment	Number of cases	Deaths	Case mortality per cent
Sulphathiazole	51	10	19·6
Sulphathiazole cum antiplague serum ..	60	12	20·0
Total ..	111	22	..

Cases with plague septicæmia at the commencement of treatment

Treatment	Number of cases	Deaths	Case mortality per cent
Sulphathiazole	18	9	50·0
Sulphathiazole cum antiplague serum	25	8	32·0
	43	17	..

Summary of Results of all Five Field Trials

In addition to the three field trials reported here, we had reported two more trials in the Annual Reports for 1936 and 1938. It would serve a useful purpose to give here a combined summary of the results of all the five field trials.

All cases

Treatment	Number of cases	Deaths	Case mortality per cent
Antiplague serum	157	37	23·5
Sulphapyridine	122	33	27·0
Sulphathiazole	230	48	20·8
Sulphathiazole cum antiplague serum	60	12	20·0
Iodine intravenously (Controls)	149	80	53·6

Case with plague septicæmia at the time of commencement of treatment

Treatment	Mild septicæmia, 1—10 colonies in 0·25 cc. of blood			Severe septicæmia, more than 10 colonies in 0·25 cc. of blood		
	Cases	Deaths	Mortality per cent	Cases	Deaths	Mortality per cent
Antiplague serum ..	29	7	24·1	42	29	69·0
Sulphapyridine ..	26	5	19·2	36	26	72·2
Sulphathiazole ..	27	4	14·8	65	36	55·4
Sulphathiazole cum antiplague serum	4	0	0·0	21	8	38·1
Iodine intravenously (Controls)	19	14	73·7	56	54	96·4

Primary Pneumonic Plague.—During these trials ten cases of pneumonic plague were treated, 6 of these, treated during the first trial of this series were definitely cases of primary pneumonic plague, the others were admitted at such an advance stage of the disease that septicæmia was present in addition to consolidation of the lungs and the diagnosis rendered uncertain. Treatment did not save any of these cases. But we feel we have not had adequate number of cases to make a thorough study.

II. Plague Vaccine

(SOKHEY AND HABBU)

With a view to produce a better plague vaccine studies into the factors which determine the immunogenic value of a plague vaccine were continued during the period under review, and the following tentative results were obtained.

(*a*) *Agar grown and Broth grown Vaccines.*—A method to estimate the number of organisms in broth vaccine (Haffkine 4 weeks' growth) was worked out. This enabled us to make agar and broth vaccines containing approximately the same number of organisms, i.e. 1000 million per cc. Agar vaccines were three-day growths of fully virulent cultures on agar incubated at 37°C. and broth vaccines were 4 weeks' growth incubated at 28°C. of the same strains. Both vaccines were heat killed at 54°C. for 15 minutes and carbolised, (0·5 per cent).

	Mouse protective dose in cc.
Agar vaccine (average of 8 experiments)	0·0065
Broth vaccine (average of 9 experiments)	0·0060

(*b*) *Method of killing Cultures.*—Besides the usual application of heat, the effect of merthiolate, sulphathiazole and alcohol (Felix) as killing agents was studied. Fully virulent strains were used.

	Mouse protective dose in cc.
Agar Vaccine—	
Heat, 54°C.	0·0065
Merthiolate, 1 in 500,000	0·0068
Sulphathiazole, 1 per cent	0·0070
Alcohol (Felix)	0·0160
Broth Vaccine—	
Heat, 54°C.	0·006
Merthiolate 1 in 500,000	0·006

(*c*) *The Relationship of the Virulence of a Strain to the Protective Power of the Resultant Vaccine.*—This question could not be tackled until we could at will reduce the virulence of a given strain to any desired extent. The method of reducing virulence used was described in the 1932–35 report (repeated sub-cultures at 37°C.). To be able to decide

this issue, it is of course, essential to use live vaccines. We would have liked to have used a live vaccine of a fully virulent strain (i.e., Mouse M.L.D. 5 organisms) but this is obviously not possible. We selected a strain of slightly lowered virulence (i.e. Mouse M.L.D. 25 organisms) and a strain of greatly reduced virulence (i.e., Mouse M.L.D. 300,000,000 organisms).

Virulence of strain	Mouse protective dose
Mouse M.L.D. 5 organisms ..	Indeterminable
Mouse M.L.D. 25 organisms ..	6 organisms
Mouse M.L.D. 300,000,000 organisms. ..	875 organisms

Immunogenicity of a strain varies with its virulence.

(*d*) *Comparison of the Immunogenic Power of different Avirulent Strains.*—Otten's (Tjiwidej sm.) and Girard and Robic's (E.V.) are now the classic avirulent strains. These were found accidently; while we have worked out a method of making a strain avirulent at will. In a previous report we gave an account of strain 120/5H. Since then we have rendered avirulent another strain 53/H. Agar vaccines were made from three-day growths of all the strains used and they contained 1,000 million organisms per cc.

Strain	Mouse protective dose
Haffkine Institute strain (53/H) ..	0·000,000,875 cc. i.e., 875. organisms.
Otten strain (Tjiwidej sm.) ..	0·001 cc.
Girard and Robic strain (E.V.) ..	0·001 cc.

Our experiments conclusively show that it is possible at will to reduce the virulence of an organism and still retain a high degree of immunogenicity. But we cannot overstress the fact that a strain to have immunogenicity must have some virulence, if it loses its virulence absolutely it has no immunogenic property left. In the case of our strain 53/H we began with a virulence of Mouse M.L.D. 5 organisms and reduced it to Mouse M.L.D. of 300,000,000 organisms. The subcutaneous injection of that number of organisms actually produces invasive infection. This fact also bears strongly on the legitimacy of the use of live vaccines made from the so-called avirulent strains.

(*e*) *Effect of killing Avirulent Vaccine.*—Since the so-called avirulent strains are only relatively avirulent and not so in any absolute sense, we tried the effect on the protective power of the resultant vaccine, if the avirulent cultures were killed. Avirulent strain 53/H described above was used. Three-day agar growths at 37°C. were used. The vaccine contained 1,000 million organisms per cc.

Method of killing cultures	Mouse protective dose
Heat, 54°C., 15 minutes. ..	0·007 cc.
Merthiolate, 1 in 500,000 ..	0·0057 cc.

It is to be noted that whether a vaccine is made from a fully virulent strain or an immunogenic avirulent strain, such as is described in (*d*) above, once the culture is killed the protective power of the resultant vaccine falls to about the same level, cf. (*b*) above.

III. Preservation of the Virulence of Plague Cultures

(SEAL AND HABBU)

To overcome the difficulties of maintaining the virulence of plague cultures the method of drying from the frozen state was studied. Of the various substrata used, namely, fat free milk, inactivated normal horse, rabbit and guineapig sera, various concentrations of gum acacia and combinations of gum and sera, 5 per cent gum acacia in the nutrient broth suspension of the organism gave consistent results. As high a survival rate as 50 per cent was attained and the virulence was strictly maintained. No change of virulence was noted when tested seven months after drying. Further observations are being continued.

IV. Serological Studies of Plague Strains

(SEAL)

A study of the serological differentiation of various strains representing virulent, protective avirulent, non-protective avirulent and pseudotuberculosis groups was carried out by means of agglutination and cross-agglutination, absorption and cross-absorption of agglutinins and precipitation tests using various antisera raised against the individual strains in rabbits.

Technique of agglutination and absorption of agglutinins.—The difficulty of obtaining a stable emulsion was overcome by using an 18-20 hours rabbit blood-agar culture at 37°C. and emulsifying it by means of a loop, to an opacity equivalent to Brown's tube No. 2, into a physiological salt solution strictly maintained at pH 7·2. The live suspension was ready for use immediately and autoagglutination was entirely avoided. The serum and bacterial emulsions were mixed together in equal volumes in Felix tubes of more or less uniform bore and were incubated overnight at 37°C. and the results were read using a low-power hand lens for the determination of endpoints.

For the absorption of agglutinins contained in an antiserum thick, merthiolate-killed, saline suspension of the organism grown on blood agar for 48 hours at 37 C. was used. This thick emulsion was added to make 1 : 5 dilution of the antiserum to be absorbed and incubated for 4 hours at 37 C. and then left in the cold (4-6°C.) for at least 48 hours, shaking several times within that period. Further amount of emulsion was added, if necessary, and the process repeated till the agglutinin against the particular strain was completely absorbed. The absorbed serum was finally adjusted to 1 : 10 or 1 : 12·5 dilution with 0·85 per cent NaCl solution and made organism free by centrifuging in cold at high speed in an angle centrifuge.

Results.—Altogether 16 strains and 16 antisera were used and more than 2,000 sets of agglutination performed. Although the work is still being continued, certain trends are clear from the results so far obtained. It shows that the plague bacillus possesses at least two antigenic components one specific to the plague bacillus and the other common to pasteurella group. The test qualitatively differentiated the different groups of organisms mentioned but did not yield quantitative results to measure the antigenic differences between the different strains.

Peculiarity of agglutination reaction of plague strains.—It was observed that the antisera against virulent plague organisms agglutinate the homologous strains to a low titre e.g., 1:400-800 while the same sera against the non-protective avirulent or pseudotuberculosis strains show much higher titre e.g., 1:3200-5000. This anomaly requires to be explained. An investigation in this line is proceeding.

Precipitin reaction.—The above antisera, both absorbed and unabsorbed, were utilised for precipitin test against the specific protein, P_I (reported elsewhere). This specific substance was isolated in a purer form than before by using a protein-free liquid medium for the growth of the bacillus. Various dilutions of the antigen were mixed with the serum in equal volumes and the mixtures were incubated for 2 hours at 37°C. followed by keeping in the cold (4-6°C.) overnight. The results were read next morning by means of a low-power hand lens.

The results obtained indicated that the precipitin reaction carried out with specific protein as an antigen was more distinctive than the agglutination reaction. By this test the various plague strains could be broadly divided into two groups which correspond to the protective and non-protective groups of strains selected. Among the protective groups the only difference between the virulent and avirulent strains was found in the titre of the reaction. The antisera against the virulent strains precipitated the specific antigen to a higher titre than those against the avirulent strains, the corresponding agglutination titres being either the same or lower than those of the latter. Also, by using the various absorbed sera for precipitin test it was found that the avirulent non-protective strains had failed to absorb the specific antibody present in the antisera against both virulent and avirulent protective strains.

V. Isolation of Specific Soluble Substance from Plague Bacillus

(Seal and Mukherji)

It was reported in the Annual Report of 1938 that the specific fraction of immunising value in the Haffkine plague vaccine was a protein. This active fraction was isolated from the supernatant fluid of plague vaccine prepared in the acid-digested mutton broth which also contained other protein fractions of non-specific character. It was therefore necessary to use a protein-free medium for isolating the specific fraction in a pure

form. At first a gelatin hydrolysate medium showing no precipitable protein was used and now it has been replaced by the casein hydrolysate medium reported elsewhere.

Method.—The supernatant fluid of the plague cultures made in the gelatin hydrolysate medium and incubated at 28°C. for 4 weeks, was made bacteria free by Seitz-filtration. This filtrate was fractionated by precipitating the protein with different concentration of Na_2SO_4 at a constant temperature of 37°C. The first fraction P_I was obtained at 33 per cent concentration of Na_2SO_4; the second fraction P_{II} was obtained by raising the concentration of Na_2SO_4 in the resultant filtrate to 50 per cent level and after its removal the third fraction P_{III}, very small in amount, was obtained by raising the sulphate concentration to 66 per cent. At full saturation of the filtrate after the removal of the above three fractions practically no precipitate was obtained. Only fractions P_I and P_{II} which constituted the bulk of the precipitate were further purified by repeated solution of the precipitate in 0·85 per cent saline and reprecipitation with respective concentration of Na_2SO_4, followed by dialysis in the running cold water. Finally, the fractions were dried in the cryochem apparatus.

Character of the fractions.—Chemical studies of the fractions P_I and P_{II} showed that both were Molisch and biuret positive and gave positive test for phosphorous. They, however, differed in their N content. Serologically, the fraction P_I was highly active. Its precipitin-titre against a potent antiplague horse serum, H_{12} unconcentrated was 2,000,000 and the quantitative equivalent zone for 0·05 cc. of the serum was 0·35 mg., the corresponding figures for the fraction P_{II} being 200,000 and 1·6 mg. respectively. The possible correlation between the quantitative precipitin-N and the protective power of antiplague horse sera at various stages of immunisation against plague culture has been noted under the " standardisation of antiplague sera ". Further work on the isolation and purification of this specific substance by using casein hydrolysate medium is in progress.

VI. A Synthetic Casein Hydrolysate Medium for the Growth of P. pestis

(Seal and Mukherji)

As the use of a protein-free medium was considered essential for the isolation of pure specific protein from the cultural filtrate of plague bacillus the acid hydrolysate of casein, as originally developed by Mueller (1939—*J. Immunol.*, *37*, 103), was thoroughly studied and modified to suit the conditions necessary for a good growth of *P. pestis*.

It was found that H_2SO_4 used for the digestion of casein could be more profitably neutralised by $BaCO_3$ instead of $Ba(OH)_2$ used by Mueller. The negligible amount of precipitable protein that appeared in the final base after full saturation with Na_2SO_4 could be removed by a light treatment with charcoal. The effects of the essential and

supplementary nutrients, e.g. N, P, Fe, Ca, Mg, cystine, tryptophane, liver extract, etc., were also studied. The medium which is now practically standardised has given better growth of plague bacillus than the acid-digested mutton broth employed for the preparation of plague vaccine. This medium has also been found to be useful for the growth of many other pathogenic bacteria and is preferred to gelatin hydrolysate medium for its cheapness and availability. Furthermore, in a preliminary test, plague vaccine prepared in the medium has proved more protent than that prepared in the acid-digested mutton broth.

VII. Standardisation of Antiplague Serum

(SEAL)

Sokhey and Maurice developed an exact quantitative method for measuring the protective and therapeutic value of antiplague serum manufactured at this Institute. But the method requires a large number of mice and involves a period of about a month for each test and this, as previously pointed out, is somewhat inconvenient for assessing the level of immunity in the animals when they are undergoing the process of immunisation. It was, therefore, considered desirable to develop some suitable test-tube method, on serological lines, which could take the place of the biological method or at least serve as an index of the potency of the serum obtained from trial bleeding of horses at different stages of immunisation. Furthermore, the successful standardisation of a biological method would also offer an opportunity to find out the relationship between the phenomenon of active immunity and serological reactions.

Agglutination and precipitation tests.—During the course of the above investigation it became clear that the direct agglutination or precipitation test carried out with antigens prepared in various ways could not be used to determine quantitatively the potency of the antiplague serum as found by the biological test. But very promising results, as noted below, have been obtained by adopting the quantitative precipitation-nitrogen or agglutinin-nitrogen test designed after Heidelberger *et al.*

Quantitative precipitin-nitrogen test.—The solution of specific protein P_I (referred elsewhere) isolated from the supernatant fluid of plague culture in gelatin hydrolysate medium was used as an antigen. The amount of nitrogen in excess of the antigen-nitrogen present in the precipitate obtained at an exact equivalent level of an antigen-antibody mixture was taken as a measure of the precipitable antibodies present in the antiplague sera of horses. The following are the results of testing the available samples.

Serum No	Date of bleeding	Amount of precipitin N per cc. in mg.	Mouse protection dose in cc.
H 27 (Conc.)	....	0·94	0·17
H 21	2nd November 1941.	0·63	0·24
H 45	2nd November 1941.	0·51	0·45
H 23	2nd November 1941.	0·40	0·60
H 27	2nd November 1941.	0·37	0·40
H 44	2nd November 1941.	0·21	0·70
H 23	28th November 1941.	1·36	0·075
H 24	28th November 1941.	0·96	0·18
H 44	28th November 1941.	0·68	0·29

It will be seen that with the exception of H 27 serum of 2nd November, 1941 there exists a fair amount of correlation between the precipitable antibody-nitrogen and mouse protection dose. Further work in this direction will be pursued.

Quantitative agglutinin-nitrogen test.—A virulent plague bacillus was grown in casein hydrolysate medium for 72 hours at 28°C., killed by 0·1 per cent formalin, centrifuged and then washed several times in formol-saline in which it was finally suspended. This emulsion standardised to give not less than 0·5 mg. of nitrogen per c.c. was used as antigen. The quantity of agglutinin nitrogen present in the test antiplague serum was measured by the maximum amount of agglutinin-N absorbed by the precipitate formed when the serum was mixed with a variable amount of the antigen and incubated at 37°C. overnight followed by keeping in the cold (4-6°C.) for at least 48 hours. The following preliminary results have been obtained.

Serum No	Date of bleeding	Antibody N per cc. in mg.	Mouse protection dose in cc.
H 23	28th November 1941.	2·04	0·075
H 24	28th November 1941.	1·24	0·18
H 44	28th November 1941.	0·60	0·29
H 27	1st May 1942 ..	1·60	0·068
H 23	1st May 1942 ..	1·28	0·084

The same correlation is noted here as in the case of quantitative precipitin nitrogen test with an additional advantage that it is more simple in details, particularly, in the preparation of the antigen. The figures of antibody nitrogen are, however, higher in this test for the same serum than that of the precipitin test. This is due to the fact that in the latter case only the specific antibodies take part in the reaction whereas in the case of aggluntinin-N the non-specific part of the antigen as well as of the serum add to the nitrogen value. Further work is in progress and attempts will be made to avoid this non-specific reaction.

VIII. The Effects of Temperature and Humidity upon the Growth of early Stages of the three Indian Rat-Fleas

(SHARIF)

Further investigations on the effects of different constant temperatures and relative humidities on the growth of larvæ and pupæ of *X. cheopis*, *X. brasiliensis* and *X. astia* were continued. Recently hatched larvæ, and resting larvæ and pupæ within and without cocoons were exposed to temperatures 45·5°, 38°, 13°, 2° and 0°C. at different effective humidities.

The third instar larvæ of *X. cheopis* containing blood in their alimentary canal, when exposed to a temperature of 45·5°C. and relative humidities of 60, 80 and 90 per cent for two minutes revived after 15 minutes' exposure to a favourable temperature and humidity; but they all died within a day. On the other hand, defæcated larvæ were not so adversely affected and about 60 per cent of them bred successfully afterwards. Some of the defæcated larvæ revived even after an exposure of three minutes, but none of them showed signs of further development. Some of the defæcated larvæ even revived after four minutes' exposure. Cocoons containing resting larvæ and pupæ of all the three species were not affected by one or two minutes' exposure to this temperature, and almost hundred per cent adults emerged from them. Even four minutes' exposure did not prove completely fatal and adults emerged from a few of them. On the other hand, five minutes exposure proved totally fatal and no growth took place afterwards. In the case of naked pupæ three minutes' exposure was completely fatal.

At temperature 38°C. and relative humidities of 80 to 100 per cent larvæ of *X. cheopis* and *X. brasiliensis* did not grow and the recently hatched larvæ of the former died within two to three days and those of the latter were killed off within 30 hours. At this temperature and relative humidities of 80 and 90 per cent, some of the recently hatched larvæ of *X. astia* showed signs of growth and 3·3 per cent. of them reached the resting stage within a period of 16-20 days. No cocoon formation and pupation were possible at this temperature. At 60 per cent relative humidity all the larvæ of this species died within a day. At 100 per cent humidity they lived for 3-5 days and at 97·5 per cent humidity for 5-11 days, but they all died in the first larval instar.

Temperature 13°C. was tried to ascertain the thresholds of development of both the larval and pupal stages of these species. The failure of larvæ of *X. astia* to grow at any humidity shows that their threshold of development is either 13°C. or a little higher. On the other hand, the pupæ of this species grew at 80 to 100 per cent relative humidities and thus their threshold of development is slightly lower than 13°C. Both the larvæ and pupæ of *X. brasiliensis* and *X. cheopis* completed their development at this temperature, but there were appreciable differences in their rates of growth. The threshold of development of the larva of both the species is lower than 13°C. and that of *X. cheopis* is slightly lower than that of *X. brasiliensis*. The thresholds of development of the larvæ and pupæ of all the three species are different, though differing from one another only a little. The threshold of development of the pupæ is lower than that of the larvæ. The cocoon affords a slight protection against desiccation and unfavourable temperatures to the resting larvæ and pupæ. Bordering temperatures and humidities which are definitely fatal to the naked larvæ and pupæ allow them to develop when within cocoons.

In order to determine the effects of low temperatures on the larval and pupal stages, temperatures 2° and 0°C. with relative humidities of 80, 90 and 97·5 per cent were tried. It was found that in these stages there were slight specific differences in cold resistance. The resistance of these stages to cold was not considerable. Even an exposure of six days to these temperatures is completely fatal and thus these fleas cannot hibernate in the larval and pupal stages. The fact that these stages cannot survive the cold storage at 0° to 4°C. for six days, can be utilised for the control of these fleas when transported from one place to another through merchandize.

The unfed larvæ resisted low temperatures for a comparatively longer period than the fed ones. Five days' exposure of recently hatched larvæ of *X. cheopis* to 2°C. proved completely fatal and none of them revived when exposed to a optimum temperature and humidity, but three days' exposure was not fatal in the sense that some of the larvæ revived. Three days' exposure of the recently hatched larvæ of *X. brasiliensis* to this temperature proved totally fatal and two days in the case of *X. astia*. In the case of fully fed third instar larvæ of all the three species containing blood in their alimentary canal two days' exposure to this temperature was completely fatal. Even one day's exposure prevented further development of these larvæ, though most of them revived. On the other hand, defæcated larvæ can withstand a comparative longer exposure. Two days' exposure of defæcated larvæ of *X. cheopis* to this temperature allowed the emergence of adults from 50 per cent of them. Similarly two days' exposure of the naked pupæ of *X. cheopis* was not fatal and 11·1 per cent adults emerged from them, though growth proceeded in 75 to 89·9 per cent of them. Six days' exposure in the case of naked pupæ was completely fatal.

Temperature 0°C. proved more injurious and all the recently hatched larvæ of *X. cheopis* died totally after 3 days' exposure. Even 50 to 86·7 revivals after one day's exposure failed to grow and all of them died within two days. Recently hatched larvæ of *X. astia* were completely killed off after 2 days' exposure. The resting larvæ and pupæ of *X. cheopis* within cocoons when exposed to this temperature for two and a half hours gave 45 to 90 per cent success in breeding. A day's exposure was fatal as regards the emergence of adults is concerned, but growth, however, proceeded in 30 to 75 per cent of them. Four days' exposure to this temperature proved completely fatal in the case of pupæ and no development took place afterwards. The resting larvæ within cocoons failed to undergo metamorphosis even when exposed to this temperature for one day, showing thereby that the pupæ can withstand a longer exposure to cold than the resting larvæ.

IX. On the Nutritional Requirements of the Larvæ of the three Indian Rat-Fleas

(SHARIF)

With a view to determine the comparative nutritional value of different foods for the flea larvæ, recently hatched larvæ of *X. cheopis*, *X. brasiliensis* and *X. astia* were reared on highly milled wheat flour devoid of bran, dried horse-blood and mixtures of equal parts of flour and horse-blood and blood and yeast, at 25°C. and 80 per cent relative humidity, and corresponding changes in the rates of their growth were noted. Larvæ of all the three species cannot breed successfully on blood alone. Less than 50 per cent larvæ of these species bred successfully on flour alone, but the adults obtained were of unusually small size and less sclerotized. The larvæ of *X. astia* bred successfully in 60–112 days with a larval life of 44–80 days and those of the other two species in 41–75 days with a larval life of 24–59 days. The rate of growth was not simultaneous and after 41 days all stages of development from a first larval instar to an adult were found. The larvæ died in different stages of growth. The marked disparity in the growth of larvæ clearly suggests some favourable change in the food, which is responsible for the successful breeding of some of the larvæ. The only apparent change noticed was the presence of fungus growth on the dead larvæ. This fungus growth had evidently served as an accessory food for the larvæ. It is, therefore, evident the wheat flour by itself has not got sufficient nutrutive value for these larvæ, and the association of micro-organisms like yeast, bacteria or fungi with food is essential. The successful breeding of the flea larvæ on flour in the presence of micro-organisms enables them to establish themselves at places far away from their original home. The larval life of *X. astia* was much longer than that of the other two species and the mortality rate was very high.

The addition of blood to the wheat flour shortened the larval life considerably, being 13-24 days in *X. cheopis* and *X. brasiliensis* and

23-37 days in *X. astia*. Almost all the larvæ grew, and many of them died in the resting stage. The percentage of successful breeding of the larvæ was low, when compared with those fed on flour, being 20·1 to 26 per cent in *X. astia* and *X. cheopis* and 10 per cent. in the *X. brasiliensis*. The absence of cocoon-formation was responsible for the high rate of mortality in the pupal and resting larval stages. The adults were highly sclerotized and bigger than those reared on flour. The nutritive value of the mixture of blood and flour is more than that of flour alone. As the mixture was less hygroscopic, it caused a high rate of mortality.

The addition of dry medicinal yeast to blood gave almost a hundred per cent success in rearing of fleas within 19-32 days with a larval life of 8-14 days. The addition of yeast increased the hygroscopic power of the food and did away the necessity of the growth of microorganisms in the food to ensure successful breeding.

It is experimentally proved that blood is not so essential for the successful breeding of the larvæ of these fleas as it is in the case of *Nosopsyllus fasciatus*. The larvæ can breed successfully without blood, but better results are obtained when blood forms the integral part of the food. In addition, it is found that association of some micro-organisms with the food ensures a more successful breeding of the larvæ.

X. On the Ecology of Plague

(Sharif and Narasimham)

Five plague infected places, viz., Barsi town, and villages Devgaon, Saundra, Bulewadi and Agalgaon, and 35 adjoining non-infected villages in the Barsi Taluka, and Dharwar town and village Narendra in the Dharwar Taluka which were plague infected, were surveyed with a view to detect plague infection in both the domestic and wild rodents from July 1940 to December 1941. In all 28,426 domestic rodents and 4,633 wild rodents from the Barsi Taluka and 3922 domestic rodents and 484 wild rodents from the Dharwar Taluka were examined. Plague in infection was detected in five wild rodents i.e., one, *Millardia meltada* and four *Tatera indica* and 38 house rodents, i.e., 36 *Rattus rattus rufescens* and two *Mus dubius* in the Barsi Taluka and one *R. rattus rufescens* and one *M. dubius* from village Narendra. The ectoparasite-and tissue-inoculation tests were found very useful in the study of the duration of the infection. Out of 403 tests tried for domestic rodents 37 were positive to plague. Out of 377 tests tried for wild rodents only 5 turned out positive to plague. Three of the positive cases were from ecto-parasites collected from the normal wild rodents. Plague infection was only detected in a few wild and domestic rodents and their fleas from the infected places.

The family Muridae in the Barsi Taluka is represented by *Rattus rattus rufescens* (Indian domestic rat), *Mus dubius* (Indian house-mouse), *Bandicota malabarica*, *Tatera indica* (Indian gerbille), *Gunomys kok*

(mole-rat), *Millardia meltada* (soft-furred field-rat), *Leggada booduga* (Indian field-mouse) and *Leggadilla platythrix*. Tree squirrels of this region belong to the species *Funambulus pennanti* and musk shrew to *Suncus cæruleus*. In the Dharwar Taluka the rodent fauna is practically the same, except that there are two subspecies of domestic rats, viz. *R. rattus rufescens* and *R. rattus wroughtoni*. On the whole the former subspecies is more common than the latter; but comparatively the latter subspecies is more abundant in a village than in a town. *G. kok* which forms about 40 per cent of the entire rodent population of the Bombay City was almost absent from the human habitations in villages and towns of both the talukas, and is only found in the open fields.

The following species of ectoparasites are found on the rodents of the Barsi Taluka :—

I. Order Siphonaptera (Fleas).—(1) *Xenopsylla astia* (mostly on wild rodents) (2) *X. cheopis* (exclusively on domestic rats); (3) *Echidnophaga gallinacea* (sometimes on domestic rats); (4) *Ctenocephalides felis* subspecies *orientis* (rarely on domestic rats).

II. *Order Anopleura* (*Lice*).—(1) *Polyplax spinulosa* (exclusively on domestic rats); (2) *Polyplax stephensi* (exclusively on *T. indica*); (3) a species of louse which appears to be new (exclusively on *M. meltada*); (4) *Pediculus humanus* (only one nymph was found on a domestic rat).

III. *Order Hemiptera* (*Bugs*).—(1) *Cimex hemiptera* (occasionally on domestic rats); (2) *Tritoma rubrofasciata* (two nymphs once on a domestic rat from a plague infected house).

IV. *Order Acarina* (*A*) *Superfamily Parasitoidea* (*Mites*).—(1) *Dermanyssus muris* (exclusively on domestic rats), (2) *Laelaps echidninus* (very common on *M. meltada*), (3) *L. nuttalli* (on *T. indica*). (B) *Super-family Ixodoidea* (Ticks).—(1) *Rhipicephalus sanguineus*, (2) *Hyalomma* (*Hyalommina*) *hussaini* and *Hæmaphysalis bispinosa* (common on both domestic and wild rodents).

The ectoparasites found on rodents in the Dharwar Taluka are practically indentical to those found in the Barsi Taluka, except that *X. brasiliensis*, *P. irritans C. felis* are common in the domestic premises. Free wandering cat, dog and human fleas were met in such large numbers in some houses and gullies in October, November and December that it was impossible to avoid them. The percentage of *X. brasiliensis* varied 40·2 to 48·3 on Dharwar town rats; but the percentage of its infestation on Narendra rats was only 7·2 to 10·1. The factors controlling the irregular distribution of the three species of the genus *Xenopsylla* are under study.

The examination of the ectoparasites of both the wild and domestic rodents has led to the conclusion that *X. cheopis* is predominantly found on the house-rat and musk shrew, and *X. astia* is occasionally found on them. On the other hand, *X. astia* is exclusively found on the wild rodents. *T. indica* harbours the largest number of fleas, with a flea

index, varying from 8·6 to 53, *G. kok* is next to it with a flea index seldom exceeding 6. *M. meltada* has the least number of fleas with a flea index less than one. Other ectoparasites like lice and mites are specific to their hosts. Advantage of this fact was taken to determine the degree of close association of wild and domestic rodents in these parts. It was found that the proportion of *X. astia* increases on domestic rats collected from houses bordering the fields. It was absent from domestic rats from the centre of the town; but it predominated on domestic rats in farm houses. Similarly the presence of other ectoparasites of wild rodents like *P. stephensi*, *L. echidninus*, and *L. nuttalli* in very small numbers on domestic rats, especially during the rainy season, shows their close association with wild rodents. Some of the collections of *M. meltada* which, as a rule, does not harbour fleas had a few specimens of *X. cheopis*, showing thereby that the rodents of this species either sometimes venture into the villages or they pick up fleas scattered by the domestic rats that leave a village for their extra-mural excursions. There is, however, some evidence to show that domestic rats that leave a village during the epizootic scatter fleas on their way. In villages Devgaon and Saundra, when the epizootic was in full swing the rats collected from the houses in the villages showed an unusually high flea index of about 20, while those trapped from the huts set up as the result of evacuation had very few fleas. The occasional presence of a very few specimens of fleas that cannot establish themselves on rodents of a locality tends to the conclusion that scattering of fleas through grain merchandize, human agency, etc., is going on in nature, and possibly it plays a part in the dispersal of plague.

The study of burrows of the three wild rodent species provides a reasonable explanation for the difference in their flea index. *M. meltada* usually lives in a straight burrow which hardly goes more than 9 inches deep into the soil and is open at both ends. As the burrow is not deep, the direct heat of the Sun raises the temperature of the nest and keeps it hot and dry. This makes it impossible for any flea to breed in the nest of this rodent. The burrows of *T. indica* go as deep as 40 inches and they have a tortuous course. The heat of the Sun cannot penetrate this depth and ventilation is very poor on account of the tortuous course of the burrow. The temperature in the nest can only be lower and humidity higher than those of the atmosphere. The temperature and humidity of such deep burrows are least affected by atmospheric changes. They remain almost constant with slight seasonal variations at such a depth. The nest of *T. indica* is thus an ideal place for breeding of fleas, and consequently it is always full of fleas, and as the result of that this species always harbours the largest number of fleas. The burrow of *G. kok* is intermediate between the two burrows. The nest is found at a depth of 12 to 18 inches but the course is such as to encourage ventilation. It is not so favourable for breeding of fleas as that of *T. indica*. Consequently, very few fleas are found in it. It is reasonable to suggest that flea index of these rodents bears some relation with the nature of their burrows. On the whole, there was slight diminution in the flea infestation of *T. indica* during hot months; but it was observed

that the flea infestation was directly proportional to the depth of a burrow during these months.

The increase in the flea index of the domestic rats is governed by three important factors, which work independently of one another. Firstly, the climatic factor controls the increase and decrease of fleas on rats. There was a considerable fall in the gross flea index of all the non-infected villages from 15th March to 15th July, when the atmospheric climate was hot and dry. In April the flea index was less than half of what it was in February. Secondly, the storage of grains in the premises increases the flea population, on account of the abundance of food for the rats and the flea larvæ. Thirdly, the existence of plague infection in the premises increases the flea population enormously. The tremendous increase in the flea population observed in the infected premises is the result of a plague epizootic rather than a cause for it. Even the musk shrews showed a gradual increase in their flea index during the course of an epizootic. In view of this, it is reasonable to suggest that a large number of fleas, many amongst them plague infected, are let loose as the result of a plague epizootic, and their transport from one place to another, through human agency, grain merchandize and wandering animals, disseminates plague.

There is another factor which plays a very important part in the ecology of plague. The duration of life of the infected fleas is very short, especially in the warm and dry months, and they can only be transported through very short distances accompanied by a high rate of mortality amongst them. It is probably for this reason the progress of plague is very slow and uncertain.

From the standpoint of plague control, an immediate destruction of the tremendous flea population in a plague infected premises is essential, so as to prevent its dispersal. In this connection the destruction of musk shrews is very desirable. They may be very useful animals otherwise; but they play a very important part in the dissemination of plague amongst the house rats of a locality, by acting as a passive carrier of infected fleas. Their wandering habits from house to house are well known. Even they undertake long excursions from village to village and they have actually been caught along with their young ones from burrows in the fields about a mile and a half away from any human habitation.

The total flea population of a premises is composed of three components: (1) those found on the body of a host (2) those found in its lair or nest and (3) free wandering fleas. These three components are subjected in nature to three different sets of climatic conditions: those of (1) the room, (2) the burrow and (3) the atmosphere. The climatic conditions of the atmosphere undergo considerable diurnal and seasonal fluctuations, those of the rooms less, and those of the burrows the least. The intensity of flea infestation of wandering rats depends upon climatic conditions of a room, which account for the seasonal

variation in their flea index. The micro-climate of a rat-burrow, especially a deep one, undergoes only slight seasonal variations, and consequently the breeding of fleas in them goes on throughout the year. It is for this reason the flea index of house rats by middle of July, immediately after a few showers, suddenly increased. The free wandering fleas are controlled by the climatic conditions of a room and those of the atmosphere. During cool and wet months, they are abundant in rooms and can remain on the human body for a long time, whilst they cannot do so during hot and dry months. From epidemiological viewpoint only the free wandering fleas are important. The disappearance of plague during hot and dry months is due to the behaviour of adult fleas towards climatic conditions rather than to their decrease in population. During these months the infection is only confined to burrows, and it moves from burrow to burrow without causing any severe plague epizootic and epidemic. It is suggested that burrows in some of the grain cellars well protected against heat, which are found in the Barsi Taluka and elsewhere enable the infection to tide over the unfavourable season.

Infection amongst wild rodents have been detected in the Barsi Taluka on eight different occasions. In seven cases *T. indica* was involved. In all instances the wild rodents were caught very near the infected villages at the height of infection in them. The maximum distance from the village from which the infected fleas were recovered from *T. indica*, was four furlongs. In view of the meagre data, it is not possible to say anything definite about their role in the ecology of plague. The fact that *T. indica* is experimentally susceptible to plague and is infested with *X. astia* to the exclusion of other fleas militates against the idea that this species serves as carrier of plague during the off-season. But, so far, there is nothing to prevent this species from playing some part in dissemination of plague in nature. It has a migratory habit and lives in colonies of about 10 or more individuals and harbours excessive numbers of fleas throughout the year. These factors favour the dissemination of plague through its agency.

On account of the total absence of plague infection during the last five months of the survey, and its subsequent disappearance from the Barsi Taluka, it is suggested that infection was introduced into the villages of this taluka by importation. Both the survey of the villages and the study of progress during the past few years tend to the conclusion that big grain trade centres are chiefly responsible for the spread of infection. The infection, when once introduced, is present more or less throughout the year either in the same village or other in the neighbourhood, in a mild epizootic form, which, when the climatic conditions become favourable, flares up into an epidemic. The grain trade centres receive the infection from these villages through imported fleas along with grain and cause the dissemination of the infection, mainly through grain, to other villages. In this act the big grain centres themselves usually do not suffer much. A large population of the rodents in these centres are highly immune to plague, so much so, that

the imported infection only smoulders amongst the sparsely scattered susceptible rodents, as long as possible, without flaring up into a severe epidemic. The comparative susceptibility of the domestic rodent population of a place plays a very important part in the dissemination of plague. Barsi Town twice got infected during the period of survey; but the domestic rodents of the town being immune, it did not lead to any serious epidemic. Latur and next to it Barsi Town are the chief distributing centres of plague in this area. It appears that the idea that plague is more a rural problem is fallacious.

In view of the experience gained, so far, in the infected villages, it is suggested that poisoned baiting, as is practised at present for a few days in plague infected villages cannot serve any useful purpose. A thorough fumigation with calcium cyanide compounds of all the houses in an infected village and burrows in them will go a long way to stop plague and prevent its dispersal. In order to make poisoned baiting a useful anti-plague measure, it is absolutely essential that it should be practised for a long time especially during off season in villages which are likely to carry over the infection. Evacuation has saved many lives in the villages surveyed, where epizootic was severe. It is strongly recommended that thorough fumigation of an infected village accompanied by evacuation and mass inoculation should be practised.

In those grain centres that often show infection on a small scale, anti-rat measures such as baiting, trapping and fumigation should be practised intensively throughout the year, and grain godowns in such places should receive special attention. Imported grains from plague infected villages should be fumigated or exposed to sun or heat before they are taken into grain trade centres.

XI. Synthesis of New Sulphanilamide Derivatives

(Ganapathi, Delivalla and Shirsat)

In continuation of the work reported last year, more than fifty heterocyclic derivatives of sulphanilamide were synthesised and the therapeutic effect of many of them tested in experimental hemolytic streptococcus, pneumococcus (type I) and plague infections in mice. These derivatives are of acridine, thiazole, thiazoline, thiodiazole, barbituric, uracil, alloxazine and pyrimidine groups. As a rule, those compounds with substituents at the amino radical of sulphanilamide were very little or not at all active. Compounds of outstanding value are obtained among the thiazole and pyrimidine derivatives with the heterocyclic ring substituted at the sulphonamide radical of sulphanilamide.

Since sulphathiazole has been found to be possessed of remarkable therapeutic properties, many derivatives of sulphathiazole with further substituents at the positions 4 or 5 or both of the thiazole ring and also at the sulphonamide grouping, were synthesised to try whether even better compounds could not be produced. Sulphathiazole derivatives

with alkyl substituents at the position 5, wherein the alkyl grouping ranged from ethyl to amyl, were synthesised and tested. While the 5-ethyl and 5-isopropyl derivatives were quite active, the 5-butyl and 5-amyl homologues were inactive. A series of 4-methyl-5-alkyl derivatives of sulphathiazole, with the alkyl grouping varying from methyl to hexyl, were synthesised and all of them were found to be therapeutically inactive. It is clear that substitutions at the positions 4 and 5 of the thiazole ring in sulphathiazole destroys the activity Derivatives with an additional carboxylic acid grouping in some of the alkyl compounds, were of diminished therapeutic activity and the toxicity also appears to be increased.

Following the successful application of sulphanilamide in the conquest of many bacterial infections, drugs of this group are also being tried for the treatment of infections of the cholera-typhoid dysentry group, which as a class differ from others in being mainly localised in the intestines. An ideal drug for the treatment of these infections should, besides possessing strong bacteriostatic or bactericidal action against the organisms remain localised in the intestines with very little of it being absorbed into the blood stream. Though sulphathiazole possesses very strong bacteriostatic action *in vitro* against these organisms, it is very quickly absorbed from the intestines into the blood stream. In trying to obtain derivative of sulphathiazole which will not be so quickly absorbed from the intestines and at the same time the anti-bacterial action will be retained, many sulphathiazole derivatives with alkyl (varying from methyl to hexyl) and substituted alkyl (B-hydroxyethyl, acetonyl, carebethoxymethyl, etc.) groupings substituted at the sulphonamide radical were synthesised. These derivatives, being insoluble in alkali were expected to be poorly absorbed from the intestines. Of these derivatives, only the methyl derivative was found to be therapeutically active while all the others were inactive.

In the course of the attempts to synthesise 2-sulphanilamidopyrimide (sulfadiazine) and derivatives by an alternative route, sulphanilyguanide was condensed with ethyl formylacetate and also ethylacetoacetate to yield respectively 2-sulphanilamido-6-hydroxypyrimidine and 2-sulphanilamido-4-methyl-6-hydroxypyrimidine respectively. These two compounds were absolutely inactive and it is of interest to note that the additional hydroxyl group in these compounds completely destroys the activity of the parent compound. Many similar condensations with sulphanilylguanidine and acetylsulphanilylguanidine have also been effected.

XII. The Mode of Action of Prontosil

(Ganapathi and R. S. Rao)

It has been suggested by Trefouel *et al.* that the therapeutic action of the dye " prontosil " discovered by Domagk, is due to sulphanilamide liberated *in vivo* by reduction. To verify this, six typical dyes of this

group, of which three were therapeutically active and three inactive were fed in 10 mg. doses to mice and the sulphanilamide produced in the blood estimated. It was found that the active ones produced about 1·6 to 2·2 mg. per cent of sulphanilamide while the inactive dyes produced only traces. The rates of reduction of these dyes in the test tube with magnesium powder or cystein hydrochloride do not explain the results. This very strongly supports the hypothesis of Trefouel *et al.*

XIII. Action of Sulphanilamide Derivatives in Experimental Streptococcal and Pneumococcal Infections in Mice

(R. S. Rao and Ganapathi)

About fifty sulphanilamide derivatives of various types synthesised have been tested in experimental hemolytic streptococcal and (type I) pneumococcal infections in mice according to the technique described previously. All compounds with substituents in the amino group as the thiourea, azodyes and heterocyclic derivatives of the thiazole, thiazoline acridine and quinoline were very little or not at all inactive. 7-N^1-Sulphanilamidoalloxazine 4-N^1-Sulphanilamidouracil and 5-N^1-Sulphanilamidobarbituric acid were inactive. Sulphanilylguanidine possessed considerable antipneumococcal but very little antistreptococcal effect. 2-N^1-Sulphanilamido-5-methylthiodiazole was less active than Sulphathiazole. 2-N^1-Sulphanilamido-4-methyl pyrimidine in 5 mg. and 10 mg. doses showed almost the same antipneumococcal effect as Sulphathiazole in 20 mg. doses.

As a result of testing the series of compounds many interesting features connecting the chemical constitution and chemotherapeutic action have come into light.

XIV. Sulphathiazole in some Experimental Bacterial and Virus Infections

(R. S. Rao and Ganapathi)

Following the successful application of sulphathiazole in the treatment of experimental streptococcal and pneumococcal infections in mice, the therapeutic effects of this drug in experimental *B. typhosum*, *V. cholerae* and fixed rabies virus infection in mice and vaccinia virus infection in rabbits have been investigated.

B. typhosum.—A strain of *B. typhosum* isolated from blood of a severe case of typhoid was passaged serially through mice until it acquired sufficient virulence for mice, such that 100 million organisms given intra-peritoneally produced death within 48 hours. The mice used were of the Haffkine Institute inbred strain, six weeks old and weighed between 15 and 20 grams. The drug was administered in 20 mg. doses, in the usual way, soon after infection, ten hours later and then twice daily for three more days. It was found that sulphathiazole protected 11 out

of 16 mice. Tests put up with sulphanilamide and sulphapyridine for comparison showed that the former protected 12 out of 16 mice and the latter 11 out of 16 mice. The results certainly justify a careful clinical trial of these drugs in typhoid. The clinical data reported so far with sulphanilamide and sulphapyridine are too scanty for a proper evaluation of these drugs in this infection. It is to be emphasized, however, that to obtain best results treatment with these drugs should be started as early as possible and an adequate blood concentration of about 5 mg. per cent of the drugs maintained throughout the course of the infection.

V. Cholera.—An Inaba strain of *V. choleræ* was passaged serially through mice so that about 875 million vibrios killed 6 weeks old mice in 24 hours. The infected mice were treated with sulphathiazole and also with sulphanilamide and sulphapyridine for comparison. The results showed that all the three drugs were of no value in the treatment of infection of mice with *V. choleræ*, all the mice dying within 24 hours. But the infection produced in mice does not resemble that in human beings.

The bacteriostatic action of sulphanilamide and sulphathiazole on *V. choleræ in vitro* was then tested. While sulphanilamide up to a concentration of 1 : 5,000 showed no bacteriostasis against an inoculum of 500 per cc., sulphathiazole in a concentration of 1 : 30,000 and against an inoculum of 500 per cc. showed some bacteriostasis (growth appearing after 6 days) and in a concentration of 1 : 10,000 no growth appeared at all. Against an inoculum of 5,000 per cc. a concentration of 1 : 10,000 of sulphathiazole showed bacteriostasis for 5 days while 1 : 5,000 of the drug permanently inhibited the growth. The other interesting finding is that this bacteriostatic action is reversed by the addition of para aminobenzoic acid, which has no growth stimulating action on *V. choleræ*. The behaviour of this organism *in vitro* according to the theories of Woods, Fildes, etc., regarding the mechanism of action of the sulphanilamide, is hardly distinguishable from that of the streptococcus or pneumococcus both of which respond to the treatment with sulphathiazole. The possible reason for the difference in the behaviour of *V. choleræ* in vitro and in vivo is being investigated.

Fixed Rabies Virus.—Three weeks old mice were injected intramuscularly into the lower third of gastro-enemius muscle with one intramuscular lethal dose of fixed virus, and sulphathiazole was administered as usual. The result was a complete failure of the drug to control the infection and all the mice died of paralysis within 15 days.

Vaccinia Virus.—Three rabbits weighing between 1·25 and 1·5 kg. were used in the experiments. The rabbits were infected with vaccine lymph on the shaved surface of the back and then treated with sulphathiazole, one by mouth and the remaining two parenterally with the sodium salt of the drug. The treated rabbits showed moderate

" take " after 72 hours while the untreated rabbits showed a typical " take " in 72 hours demonstrating that the drug has little or no action on vaccinia virus infection.

XV. Synthesis of N^1-Substituted Sulphanilamides

(RAJAGOPALAN)

With the object of ascertaining the relative anti-bacterial merits of typical N^1-substituted sulphanilamides, a series of compounds derived from representative carbo-(benzene, diphenyl, ether, phenanthrene, chrysene) and hetero-cyclic (coumarin, dibenzofuran, carbazole, isoquinoline, phenanthridine, acridine, indazole, triazole, benzotriazole, indotriazine and thiazoline) ring-systems were prepared. Not counting the related intermediates, 24 final sulphanilamides were obtained. About 8 of them were investigated as to their usefulness in experimental bacterial infections with particular reference to plague in mice. With the exception of one of them, " Sulphathiazoline ", all the compounds tested appear to be inactive. The encouraging results obtained with sulphathiazoline in preliminary animal experiments warranted further intensive elaboration in this series and was followed up by synthesis of a few derivatives, which are now being subjected to pharmacological study.

XVI. Therapeutic Action of Sulphanilamide Derivatives in Experimental Plague Infection in Mice

(GANPATHI, DIKSHIT AND SOKHEY)

The experimental plague infection in mice has proved to be eminently suited to accurately detect and compare the therapeutic activities of the drugs of the sulphanilamide group. From 1939 up to January 1942, sulphanilamide and fifty-one of its derivatives have been tested. The results obtained with two of them, sulphapyridine and sulphathiazole, have already been described in the previous report and those with the rest of the compounds are summarised in the following.

Sulphanilamide (when fed in 20 mg. doses twice daily for 10 days) saved 50–90 per cent of the mice when the treatment was commenced immediately after the infection or 24 hours after; delaying the commencement of the treatment by 48 or 72 hours resulted in the saving of only 10–20 per cent of the animals. Para aminobenzoic acid fed along with sulphanilamide completely nullified the therapeutic effect of sulphanilamide.

Sulphanilamidobenzene and seven of its derivatives with the nitro, amino, dimethylamino, acetyl, carboxyl and sulphonamido groupings in the second benzene ring were tested commencing the treatment immediately after infection. Of these compounds, the two nitro derivatives (meta and para) (in 10 mg. doses) showed activity comparable to sulphanilamide, while the rest just prolonged the lives of the animals as compared with the controls.

Sulphanilylguanidine (sulfaguanidine) (fed in 20 mg. doses twice daily) saved only 10 to 20 per cent of the animals when the treatment was started same time as the infection or 72 hours after. The sulphanilamido derivatives of diphenylether, phenanthrene, carbazole and acridine were inactive.

Twenty derivatives of sulphathiazole with alkyl and substituted alkyl substituents at the positions 4 and or 5 of the thiazole ring and also sulp onamide grouping have been tested. 4-Methylsulphathiazole was about as active as sulphathiazole. The introduction of an additional carboxylic grouping into this compound at the position 5 or the methyl grouping resulted in the increase of toxicity and decrease of therapeutic activity. Of the 5-alkyl derivatives of sulphathiazole, wherein the alkyl radical ranged from ethyl to amyl, the ethyl and isopropyl compounds each (when fed in 20 mg. doses twice daily) saved 8 out of 10 mice when treatment was started immediately after infection and 6 and 5 respectively out of 10 mice when the treatment was started 48 hours after infection; the butyl and amyl homologues were inactive. Of the nine (N^1) sulphonamide substituted derivatives of sulphathiazole, wherein the substituent varied from methyl to hexyl and also contained additional hydroxy, keto and carbethoxy groupings, only the methyl derivative was therapeutically active which saved 5 out of 10 mice when the treatment was commenced immediately after infection or 48 hours later. 4-Methyl-5-alkyl derivatives of sulphathiazole, wherein the alkyl groupings were methyl, butyl, isoamyl and hexyl, were all inactive.

The structural isomer of sulphathiazole containing the thiazole ring substituted at the amino grouping of sulphanilamide, was inactive; two more derivatives of this compound were also inactive.

Sulphathiazoline (when fed in 20 mg. doses twice daily) saved 7 out of 10 mice when the treatment was started at the same time as the infection but only 2 out of 10 mice when treatment was delayed by 48 hours. The corresponding N^1-methyl derivative was more toxic and less active.

2-Sulphanilamido-4-methylpyrimidine (when fed in 5 mg. doses twice daily) saved all mice when treatment was started 24 hours after infection. It saved 3 out of 10 mice (in 10 mg. doses) when the treatment was commenced 72 hours after infection. 2-Sulphanilamoido-6-hydroxypyrimidine and 2-sulphanilamido-4-methyl-6-hydroxypyrimidine were devoid of activity.

The comparison of these results with those obtained with the same compounds against the streptococcus and pneumococcus infections in mice reveals a fairly close correspondence between them. The compounds found to be inactive in plague infection (i.e., those cases wherein the average survival time of the treated mice did not differ from that of the controls) are inactive on the other two infections also. The compounds which show definite protection in plague infection when treatment was started 48 or 72 hours after infection, possess very good therapeutic effect in pneumococcus as well as the streptococcus infection.

The compounds which show good therapeutic effect in plague infection when treatment was started immediately or 24 hours after infection but not later, show good protection in streptococcus infection but not in the pneumococcus infection. Thus, on the basis of the results obtained with the compounds in the plague infection, it appears possible to form an idea of their probable therapeutic effects in the streptococcus and pneumococcus infections.

XVII. Some Observations on Sleep

(DIKSHIT)

The work on the problem of "Sleep" was summarized in the Presidential Address given before the Physiology Section of the Indian Science Congress. It was pointed out that because acetylcholine appears normally in the brain and because it can produce sleep if applied directly to the brain centres its claim as a sleep producing hormone was worth considering. The points in favour of this claim were that "Sleep" is a parasympathetic phenomenon and acetylcholine is intimately concerned with parasympathetic phenomena, and the fact that some of the physiological changes seen during sleep such as effects on respiration, circulation and sweat secretion can be reproduced by application of acetylcholine to the hypothalamic centres. Direct and indirect evidence in favour of this hypothesis was discussed and a case made for consideration of acetylcholine as a sleep producing hormone.

XVIII. Sulphathiazole in Monkey Malaria

(DIKSHIT)

The action of sulphathiazole in malaria was investigated in rhesus monkeys infected with *Plasmodium Knowlesi*. After the number of parasites in the peripheral blood had reached a predetermined level, the drug was administered by mouth in doses varying from 0·5 g. given once to 0·5 g. given twice a day for three consecutive days. It was found that a single dose of 0·5 g. was sufficient to make the parasites disappear from the peripheral blood and a dosage not larger than 3 g. given over a period of three days brought about a radical cure. As Knowlesi infection in the rhesus monkey invariably ends fatally unless adequately treated, the uniformly successful treatment of this infection by sulphathiazole was thought to be very significant. Further, the comparatively large dosage of 1 g. a day for 3 days did not produce any signs or symptoms which could be attributed to the toxic action of the drug.

XIX. Spleen Extract and Malarial Immunity

(DIKSHIT)

The fact that the spleen plays such an important part in immunity in monkey malaria suggested that humoral antibodies may be present in spleens removed from "immune" animals. Extracts of "immune" spleen were tried by some workers before to see whether they could confer any immunity towards malarial infection in monkeys. Such extracts, however, proved to be too toxic to enable an adequate dose

being administered. In order to get over this difficu'ty of toxicity the proteins of the spleen were separated and injected intravenously and intraperitoneally in normal monkeys. It was found that extracts of spleens removed from normal monkeys failed to confer any immunity o i rhesus monkeys against Knowlesi infection, while some preliminary experiments suggest that extracts of spleens removed from " immune " monkeys could confer a certain degree of protection against this infection. Further experiments in this connection are in progress.

XX. Pharmaco'ogy of some New Sulphanilamide Derivatives

(PATEL)

An investigation of the pharmacological action of two sulphonamides N^1-Methyl sulphathiazole and 2-N^1-sulphanilamido-5-ethylthiazole, prepared by the Chemotherapy Department has been undertaken. The absorption and distribution in the body of these t o drugs has been studied and their action on different systems of the body such as the cardiovascular, gastro-intestinal, nervous and genito urinary systems has been investigated. It has been found that these drugs exert very little action on different organs of the body in doses that are likely to be employed in therapeutics.

XXI. 2-N^1-Sulphanilamido-5-ethylthizaole in Monkey Malaria

(PATEL)

The action of this sulphanilamide derivative which was prepared and supplied by the Chemotherapy Department was investigated in rhesus monkeys infected w th *Plasmodium Knowlesi.* The an mals were infected intraperitoneally and after the number of parasites in the peripheral blood had reached a level of approximately 100 parasites per 10,000 normal R. B. Cs. the drug was administered orally by a stomach tube in doses varying from 1·0 g. given only once to 1·0 g. given once a day for three consecutive days. It was found that even a single dose of 1·0 g. given nly once caused disappearance of parasites from the peripheral blood. The question of radical cure was also investigated and it was shown that 1·0 g. dose of this drug given once a day for three consecutive days produced a radical c re in monkeys. The action of this drug was therefore found to resemble very closely the action of sulphathiazole in similar malarial infections.

XXII. Sulphathiazole in the Treatment of Wounds and Burns

(DIKSHIT)

The question of treatment of burns has again become controversial and the commonly used tann ng methods are now discredited by many observers. The use of sulphathiazole in the treatment of burns was, therefore, investigated. Burns of various degrees were produced in experimental animals and treated with sulphathiazole suspended in different mineral and vegetable oils and oils of animal origin. It was found that a suspension of this drug in cod-liver oil was the best method of applying sulphathiazole to the burns and this method of treatment produced a better result than many other methods of treatment commonly employed at present.

Similarly the use of various sulphonamide drugs in crushed and infected wounds was investigated. Crushed wounds were produced in animals under other anæsthasia and these were infected artificially with strepto and staphylococci. Sulphonamides like sulphanilamide, sulphathiazole and some other sulphanilamide derivatives were tried and it was found that sulphathiazole produced the best results.

XXIII. Immunity in Monkey Malaria

(DIKSHIT)

The problem of immunity in monkey malaria was pursued on the same lines as was described in last year's report by employing the method of cross circulation. The role played by the reticulo-endothelial cells in giving protection against malarial infection formed the chief subject of investigation. For this, the response of immune splenectomized monkeys with their R. E. cells blocked by India ink was tested after 70 per cent or more of their blood was replaced by normal non-immune blood. These experiments suggest that if the cells are so blocked the animals become susceptible to fresh infection. As replacement of 70 per cent or more of their blood with normal non-immune blood does not affect immunity of animals with their spleens and other R. E. cells intact, these experiments suggest that the cells play an important role in malarial immunity in monkeys.

XXIV. Preparation of India Ink for Intravenous Injection

(MAHAL)

Commercial India ink varies considerably in its composition and contains substances like "Shellac" which make it dangerous for intravenous injections. In order to obtain a comparatively less toxic carbon suspension of a uniform composition the following method was adopted :—

10 g. of carbon [lamp black (fuligo) of Mercks] is first cleaned and soxhleted with ether or petroleum ether to remove oily material. The solvent is completely removed and the carbon emulsified in mucilag acacia (10 per cent) to make a 5 per cent suspension. It is important to see that the gum acacia used is according to the British harmacopœia requirements. The carbon suspension is strained through several layers of muslin and stored in a pyrex glass bottle in the refrigerator. The carbon suspension is tested in cats by intravenous infusion of known quantities and noting the vasomotor and bronchomotor responses. Preparations which show a marked broncho-constriction or a marked fall in blood pressure are discarded. These reactions are probably due to the agglutination of carbon particles. Carbon suspensions which pass the test are diluted with an equal volume of sterile normal saline, boiled, cooled to body temperature and injected intravenously.

XXV. Blood Urea Clearance in Normal Indians

(S. K. GOKHALE)

Blood urea clearance values of one hundred and ten normal Indian male subjects have been examined. The following table gives the results obtained :—

Average and range of variation in normal findings of 110 healthy young Indians

	Mean cc. per minute	Maximum cc. per minute	Minimum cc. per minute	Standard deviation	Surface area square meters	Clearance corresponding to surface area of 1·73 sq. m.
Maximum clearance $\frac{UV}{B}$	40·01	52·10	28·59	±5·35	1·57	44·0
Standard clearance $\frac{U\sqrt{V}}{B}$	31·50	43·62	17·02	±5·71	1·61	33·8

Individual variation in the blood urea clearance values in some subjects has been studied and the following results obtained :—

Blood urea clearance values of seven normal subjects on different days

Clearance Values in cc.

Number of determination	1	2	3	4	5	6	7
1 ..	28·4	35·3	21·8	39·7	34·7	25·1	34·5
2 ..	35·9	31·3	24·1	35·1	32·0	27·7	31·9
3 ..	25·2	29·0	31·2	41·1	41·1	..	..
4 ..	35·4	36·0	25·0	32·9	..	..	..
5 ..	28·8	..	..	..	..	..	..
Mean ..	30·7	32·9	23·6	37·2	35·9	26·4	33·3
S. D. ± ..	4·7	3·3	0·52	3·3	4·7	..	..

XXVI. Chemical Composition of Urinary Excretion by Normal Indians

(S. K. GOKHALE)

It has been observed (Gokhale: *Ind. J. Med. Res.* 1939, 26, 675) that the urinary excretion of total nitrogen by normal Indians is less than half that of normal Americans or Europeans. It was thought necessary to have data for the content of the various chemical constituents of the urine of normal Indians, as such data is very meagre, and this study was therefore undertaken. Twenty-four hour collections of urine of one hundred and seventeen normal Indian subjects have been examined, the following table gives the results :—

Constituent	Average	Range
Volume	1,211 cc.	420 cc. — 2,670 cc.
Specific gravity	1,018	1,007 — 1,028
H. ion concentration	5·80	5·1 — 6·7
Total acidity	259 cc. of N/10 NaOH	200 — 316 cc. of N/10 NaOH
Total nitrogen	5·82 g.	3·62 — 9·84 g.
Urea nitrogen	4·62 g.	2·74 — 7·54 g.
Uric acid	0·51 g.	0·28 — 0·81 g.
Creatinine	1·246 g.	0·81 — 1·78 g.
Creatinine coefficient	22·2	17·9 — 25·8
Ammonia N.	0·22 g.	0·15 — 0·36 g.
Amino acid N.	0·26 g.	0·15 — 0·39 g.
Inorganic phosphorous	0·73 g.	0·38 — 1·21 g.
Chlorides as NaCl	9·52 g.	2·79 — 16·56 g.

XXVII. Bactericidal Power of Normal Serum and Defibrinated Blood of Experimental Animals and Man with special Reference to Pasteurella Pestis

(SOMAN)

An investigation was carried out to study the bactericidal power of serum and de brinated blood in experimental animals and human beings towards a virulent strain of *Pasteurella pestis*. The sera and blood of smaller experimental animals were pooled together while those derived from bigger animals and human beings were treated separately.

Besides, sera were tested with and without the complement. A constant amount of serum was mixed with graded bacterial suspensions and the periods of contact of the virulent organisms with the sera and blood were 4 hours, 24 hours, 48 hours and so on till 192 hours were reached, subcultures being made at the end of each period.

The sera and blood of experimental animals showed no evidence of bactericidal action against the virulent strain of *P. pestis*. The presence or absence of the complement made no difference whatsoever. The results of the horse sera and rat sera were found to be irregular. On the other hand, human sera showed definite evidence of early and rising bactericidal activity.

Twenty-five human sera were tested in this manner. The results, giving the percentage of samples giving positive bactericidal action and the average bactericidal index are given in the table below :—

Period of contact	Percentage of sera showing bactericidal action	Average bactericidal index
48 hours	8	2
72 „	48	2·25
96 „	56	2·92
120 „	64	2·7
144 „	76	3·84
168 „	76	4·8
192 „	76	5·37

The earliest period at which bactericidal activity manifested itself was 72 hours, the action being further intensified to the end of the period of 192 hours. By the end of 192 hours, a small amount of human serum as 0·8 cc. could sterilize 80 millions of the virulent organisms. Human sera in the absence or presence of complement showed decisive bactericidal effect while the defibrinated human blood completely failed to show any such evidence.

The age of the sera, like the complement, appeared to have no effect on the bactericidal activities as the sera were obtained from two to ten days previously. Sera showing varying degrees of hæmolysis failed to show any bactericidal activity. Whether defibrinated blood showing similar reaction is due to the small amount of breaking down of red blood cells is not known.

The results obtained point out the advisability of testing sera not only for 4 to 24 hours but for a longer period. It is for this reason Mackie and Finketstein failed to demonstrate natural bactericidal power in human sera against *P. pestis*.

XXVIII. A 24-hour Test (Aschheim-Zondek modification) for the Diagnosis of Pregnancy

(SOMAN)

Attempts are made to shorten the period of the classical biological tests such as that of Aschheim-Zondek or Friedman, for the diagnosis of pregnancy. Richard Kelso, in 1940, in order to eliminate certain disadvantages, following Friedman's modification, introduced albino rat as the experimental animal of choice instead of a mouse or rabbit. Rats are considerably cheaper than suitable rabbits and they are easy to handle and inject with very few mortalities.

Material for the trial was received chiefly from hospital ward patients. Within a period of a year and a half, 109 specimens of urine were tested by the present modification as well as by the Friedman test.

Four immature female white rats between the ages of 22 and 40 days were selected for each test and the technique of Richard Kelso was followed in carrying out the injections. Two rats were killed after 24 hours and two after 72 hours. Attention is paid only to the ovaries while neglecting the tubes and uterus. A reaction is considered positive when both ovaries are enlarged and hyperaemic. A negative test is indicated by small and pale ovaries.

The 24-hour and 72-hour tests on the rat compared very favourably with that of the Friedman's modification and confirmed the diagnosis of pregnancy, vesicular moles, etc., and excluded a large number of other conditions that stimulated pregnancy and its allied pathological conditions. The rapid reading of the result in 24 hours enhances its value particularly in the diagnosis of ectopic gestation. The test appears to be accurate, economic, rapid and convenient and can be suitably employed to replace the rabbit test or to supplement it wherever necessary.

XXIX. The Distribution of Typhoid Vi Agglutinins in Normal Sera with special reference to their Diagnostic Value in Typhoid Fever

(SOMAN)

In view of the paucity of published data on the subject regarding the distribution of Typhoid Vi agglutinins in normal sera, 300 samples of sera, selected at random from those submitted for Wassermann reaction were tested. The antigen used was a live suspension of Bhatnagar's Vi variant of *Bacillus typhosus*. In addition to this, a preserved agglutinable Vi suspension, obtained from Enteric Laboratory, Kasauli, was used in the earlier part of the investigation. The suspensions both live and killed were required to satisfy the criteria of sensitivity to Vi sera in high dilutions and non-agglutinability in pure O and H antisera, even in dilution of 1 in 5. Dilutions were put up in Felix tubes and Dreyer's drop technique was used, the tubes being incubated at 37°C. for two hours and at room temperature overnight.

The results are summarized in the following Table No. I. Total number of sera examined was 300.

Serum dilutions..	5	10	16·6	20	25	Total
Vi positive sera ..	17	4	1	0	3	25
Percentage positive ..	5·65	1·35	0·3	0	1 0	8·3

From the above table it was evident that nearly 92 per cent of normal sera failed to show any presence of Vi antibody even in a dilution of 1 in 5. Thus if we take a dilution of 1 in 10 as the minimum diagnostic titre for diagnosis of typhoid infection, the test would get more than a fair chance.

With this preliminary data a further investigation of Vi development was carried out in 75 bacteriologically controlled cases of typhoid fever. All the cases were uninoculated with T. A. B. vaccine. Only one single sample of serum from each patient was available for testing the presence of O, H and Vi antibodies. The technique remained essentially the same as described above except that serum dilutions ranged from 1 in 10 to 1 in 200. The majority of samples of blood were received during the second week of fever. The results are tabulated below.

TABLE II

—	Positive by "H" test	Positive by H and O test	Positive by H, O and Vi test	Positive Vi test alone	Negative by H, O and Vi test
Number of cases ..	50	70	71	46	4
Percentage	67	93	95	61	5

The results clearly demonstrate that only one case would have been missed if Vi test was not done, but 25 cases would have been missed, if reliance was placed only on Vi test. Thus 29 out of 75 cases failed to show any Vi antibodies with one single serological examination of each case.

It appears, therefore, in cases of typhoid fever uninoculated with T. A. B. vaccine and where a single serological examination is available, Vi agglutination test possesses hardly any diagnostic value and is actually inferior from that point of view, to the routine combined method of clot culture and O and H agglutination.

XXX. A Rapid Method of Staining Malarial Parasites in Thick Blood Smears

(SOMAN)

A preliminary malaria survey of the North Kanara Distr'ct by Lt. Col. R. C. Wats, provided an opportunity of examining nearly 550 blood films, thick and thin, for the presence of malarial parasites by a simple and rapid method of staining dried blood smears, devised by Field of the Federated Malay States. The thin and thick blood smears were chiefly collected from village children between the ages of four and ten at a time when they were free from malarial fever.

The technique of staining employed was as described by Field, the staining process taking not more than two seconds. The staining solution consists of an aqueous solution of brilliant cresyl blue buffered with phosphates. The outlines of the leucocytes and blood protozoa are well preserved and they stained in marked contrast against a pale smooth yellow background of the residual ha moglobin. The new process of staining not only affords a rapid means of coming to a diagnosis but the 50 u thickness of the dried blood smear gives a sixteen times concentration o the parasites.

Out of 551 thin films giemsa-stained only 98 were positive giving a percentage of 17·7. Out of 537 thick smears stained by new method only 79 were positive giving a percentage of 14·7. When both methods were considered, 114 were positive, giving a percentage of 20·7. It may be noted that the time spent on examining a thin film was never less than thirty minutes in the absence of parasites; while the time spent on examining thick blood smears stained by the new process was never more than five minutes.

All the three species of the malarial parasites could be spotted with a fair degree of accuracy and the mixed infections were found to be not uncommon. The new staining method is very valuable as supplementary to the old conventional methods for routine use.

XXXI. Saponin-Broth as a Blood Culture Medium for Enteric Group of Organisms

(SOMAN)

With an opportunity of do ng blood cultures at the bedside, presented by the Typhoid Enquiry carried out by this Institute, it was decided to try saponin broth as a blood-culture medium for the primary isolation of the enteric group of organisms, in addition to the routine use of bile media.

Saponin broth was prepared as described by Penfold and his colleagues. Patient was bled 6-8 cc., the saponin broth and the bile medium received 2 cc. each and the rest was put for clotting and obtaining the serum for Widal test. The remaining clot was cultured

in ox-bile. All the cultures were incubated at 37°C. and subcultures were made at the end of every 24 hours for five days if negative and the results were noted.

Out of 52 cases so tried, the total number of positive cultures by either method was on y 17, out of which 12 howed a growth of *Bacillus typhosus*, 4 showed growth of *B. paratyphosus* A and one gave pure growth of *B. coli*. Out of 17 positive blood cultures, there were two cases in which saponin broth culture was positive for *B. typhosus* but the ox-bile cultures of blood and clot remained sterile. But there were 8 cases out of 17, nearly 50 per cent, which were either negative .n the sapon n broth medium or were only late po.itive.

Thus for the primary isolation of enteric group of organisms, saponin broth as a medium for culture offered no advantages over the routine ox-bile media.

XXXII. Typhoid Fever in the City of Bombay

(SOMAN)

During the two years under review (1940 & 1941) free diagnostic aid to private medical practitioners for the diagnosis of cases of enteric fever was continued, except that additional help of sending out a medical officer from the Institute to take samples of blood from patients was withdrawn from the commencement of the year 1941. Thus during 1941, blood samples were collected by the practitioners themselves and were delivered at the Institute.

I. *Diagnosis of Cases.*—The procedure adopted was the same as that of the two previous years. In children where there was difficulty of getting blood, examination of urine and faeces was suggested. Thus during the year 1940, the Institute received 700 calls as compared with the 575 calls of the previous year from the city and suburbs. Besides this 495 blood samples were received from the different hospitals in the city for Widal and culture. In 1941, 375 blood samples were sent by private medical practitioners and 630 samples by the hospitals.

II. *Blood Cul ure.*—During the years 1940 and 1941 the blood was cultured in sterile Ox-bile 20 cc., instead of that in glucose broth 100 cc., otherwise the routine procedure followed remained exactly the same as that of previous year.

III. *Widal Test.*—Dreyer's technique was adopted for the Widal test. Titre of 1 in 125 was taken as diagnostic in case of "H" agglutinins and 1 in 250 in cases of "O" agglutinins.

IV. *Cultures of Faeces and Urine.*—Wilson and Blair's bismuth-sulphite agar was made for isolation of the enteric organi ms from faeces and urine of patients.

V. Results obtained during the years 1940 and 1941 are given in the two tables below :—

TABLE A

Year	Blood samples	Positive for enteric	Culturally positive	Typhoid positive	Para-typhoid A positive	Para-typhoid B positive
1940 ..	700	324	167	290	34	0
1941 ..	375	154	62	141	13	0

Of the samples sent by hospitals 142 proved positive in 1940 and 182 in 1941.

TABLE B

The following table gives distribution of 324 positive enteric cases during the year 1940

Ward	A	B	C	D	E	F	G	Suburbs
Number of cases	10	28	38	57	11	87	50	43

Wards F, G and D contributed more than half the number of cases. Amongst the suburbs, Santa Cruz and Ville Parle were the worst to suffer and together had 24 cases. Similar information of distribution for 1941 is not available.

XXXIII. A Rapid Malaria Survey of North Kanara District

(WATS)

To draw up a scheme for malaria work for the District a rapid survey was carried out during the year 1940. The area was divided into three parts and certain villages were picked up from each unit showing the varying intensity of the prevalent malarial conditions. Following small towns and villages were visited namely Yellapur, Supa, Kirwatti, Virkhol, Asangaon, Sirsi, Makkeri, Kadwad and Karwar. From each of these places, school children and other small children between the ages of four and ten were examined for enlarged spleen. Blood films thin and thick were collected from them at the same time. Adult anopheline mosquitoes were caught and collected for identification.

Thus the total number of children examined for enlarged spleens with their blood films was 538, in addition to 157 anopheline mosquitoes caught for identification.

The following are the spleen and parasite rate encountered in different villages :—

Name of village	Spleen rate	Parasite rate
1. Yellapur	30·7	28·8
2. Kirwatti	76	38
3. Supa	32·8	16·4
4. Virkhol	100	
5. Asangaon.. ..	100	44·5
6. Sirsi	24	24
7. Karwar	less than 1	less than 1
8. Makkeri	74	0
9. Kadwad	8	8

As would be noted from the above list, the spleen and parasite rate was high in Yellapur and Supa and others, Sirsi being intermediate between the two. It was lowest in the Karwar area. Plasmodium falciparum infection predominated usually but mixed infections were by no means uncommon.

Eight species of anopheline mosquitoes were identified of which only two were the most important from the point of view of malaria carriers, namely, *A. culicifacies* and *A. fluviatilis*. The other species found were as follows—*A. subpictus, A. vagus, A. barbirostris, A. pallidus, A. Jamesi, A. jeyporiensis*. Among the two known malaria carriers in this area, *A. culicifacies* was more predominant than the other. *A. fluviatilis* though met with in small numbers is a dangerous malaria-carrying mosquito, known for its house-hunting and breeding in rice-field drains, slow-flowing small streamlets and large streams with grassy edges. Herbage packing controls the breeding of this species very effectively.

The wide stretches of jungles, large amount of rainfall and abundance of rice and other wet cultivation in addition to the ignorant, poverty-stricken and scattered population would discourage the most optimistic malariologist to suggest any scheme of action even after a prolonged enquiry. It is not feasible economically to eliminate breeding places of the anopheline mosquitoes wholesale but it is recommended that three units should be organised and they should carry on the work in the Talukas of Yellapur, Supa, Sirsi which are undoubtedly malarious. The coastal zone being fairly healthy may at present be left out of the scheme. These units should not only gauge the intensity of malaria and study the ecology of *A. culicifacies, A. fluviatilis* and others, which may prove to be carriers of malaria but undertake in addition the antimalarial work which is suggested below.

1. Destruction of adult mosquitoes in village huts and cattle-sheds by method of spraying them with Pyrocide 20 or any similar preparation known to kill and eradicate the mosquitoes.

2. Quinine treatment.
3. Propoganda against malaria.

The units should also study the effect of " Herbagepacking " referred to above as it is the only economic antilarval measure known against *A. fluviatilis* breeding.

XXXIV. Leptospirosis in the City of Bombay

(M. N. LAHIRI)

It is gradually becoming evident that the incidence of leptospirosis is more common in India than was formerly believed. It seems likely that for want of laboratory studies many cases must have escaped detection. On purely clinical grounds three epidemics in the City of Bombay have been attributed to leptospirosis. The symptoms described do point to this infection, but unfortunately no laboratory studies were carried out. Further the disease does not always occur in an epidemic form, sporadic cases are quite common. The present study was undertaken to carry out a thorough laboratory investigation into the incidence of the disease in Bombay.

(*a*) *Studies on Leptospira icterohæmorrhagiæ in rats.*—The study was pursued with a view to demonstrate not only the prevalence of infection but also to isolate and identify the strains with particular reference to their serological classification. It has a bearing on the epidemiology of the disease. The following methods of investigation were used (*i*) microscopic examination of the kidney cortex emulsion by dark-ground illumination, (*ii*) inoculation into animals, (*iii*) culture of the kidney cortex, (*iv*) serological testing of the sera of rats for the presence of agglutinins and lysins. Rats from a particular congested areas of the City were trapped and examined by the above methods. In all 125 rats were examined. Microscopic examination gave 11 positive results, and by animal inoculation 5 proved positive, of these 3 were positive by the microscopic examination. Two other animals gave evidence of infection serologically. Thus 15 or 12 per cent of the rats were found to be vectors of *Leptospira icterohæmorrhagiæ*. The results of the culture of the kidney cortex were far from satisfactory on account of an intercurrent infection in most of the rats due to *Bact. pseudoasiaticum*. The observations indicated that no single method is absolutely satisfactory and therefore the importance of the combination of various methods is stressed. The infected rats all belonged to the species *Rattus norvegicus* except one which was of the species *Rattus rattus rufescens* and whose serum showed anti-leptospiral agglutinin up to a titre of 1 : 640. Five strains were isolated from the rats and they all reacted up to the full titre with anti-liptospiral serum (Classical) prepared here.

(*b*) *On the Transmission of Infection in the rat.*—Direct observations as to how the infection of pathogenic leptospirae is maintained in the rat population are lacking. Since leptospirae are excreted out in the urine of the infected rat, it has been suggested that the infection occurs

through ingestion of food contaminated with such urine. Possibly also leptospirae may gain entrance into the body through infected material coming in contact with the skin with open wounds. During the course of study a carrier pregnant rat was trapped. Strains were isolated from the mother as well as the foetuses and were found to be serologically identical. This observation therefore presented evidence in support of the natural infection of the rat through the placenta. Accordingly it is suggested that infection in the rat with *L. icterohamorrhagiæ* is likely to be maintained through the placental route.

(*c*) *Human cases of Leptospirosis.*—A study was undertaken of all cases of acute jaundice with fever at the start admitted to local hospitals. This seemed to be the best line of approach for the detection of the disease. During the period under report 72 cases were studied, of these eight proved to be cases of leptospirosis. The diagnosis in each instance has been confirmed either by the isolation of the causal organism or by serological tests. For the isolation of the organism and also for the maintenance of strains for serological tests Vervoort's medium according to Schuffner's formula was used. But certain modification had to be introduced to standardise the media to suit the local conditions. These positive cases do not, however, present a true picture of the actual incidence of the disease in the City, because all the cases might not have come to the hospitals for treatment. Moreover the disease sometimes assumes a mild form and it is extremely difficult to make a diagnosis of leptospirosis in such cases without laboratory aid.

XXXV. A Mouse Protection Test for the Assay of Tetanus Toxoid

(D. C. LAHIRI)

A method of biological assay of tetanus toxoid by testing the tolerance of immunized mice for a dose of toxin was evolved. It was found that a toxoid passing the mouse test also passed the test laid down in the British Therapeutic Substances Regulations, which measures the antitoxin content of guineapig immunized with tetanus toxoid under test. This method has the advantage over the official method in being less expensive in time and animals. Further studies on some of the different aspects of the mouse test elicited the following results :—

(*a*) The mouse is as suitable as the guineapig for toxin tolerance test.

(*b*) Mice vary widely in their response to immunization with toxoid, but a big dose of toxin such as 15 M. L. D. or more covers this variation by eliminating all excepting a less variable population, the highly immunes, in a group of 24 mice or more.

(*c*) The measurement of the potency of a toxoid with mouse test gives repeatable results within very close limits.

(*d*) There seems to be a fair degree of correlation between the mouse protection value of a toxoid and its antigenic value in man.

(*e*) A toxoid passing the mouse test can immunize man successfully.

XXXVI. Immunizing Value of Anti-rabic Vaccines

(R. S. Rao)

Lack of a suitable laboratory test has held up studies and consequent improvements of anti-rabic vaccines used. The mouse test described by Webster of the Rockefeller Institute offers great possibilities. Work was therefore taken up to master the technique and test the protective power of different anti-rabic vaccines.

In the first instance the relative merits for purposes of vaccine manufacture of two strains of fixed virus namely the "Paris" strain and the "Kasauli" strain were investigated. The results showed that the "Paris" strain was preferable to the "Kasauli" strain.

Semple's method of preparation of anti-rabic vaccine, which is the one adopted in all Pasteur Institutes in India, was compared with Fermi's method. There was practically no difference between the antigenic qualities of the two vaccines.

Vaccines from the different Pasteur Institutes in India were also tested. The results while they showed only minor differences in protective power amongst the 5 per cent vaccines, demonstrated considerably less immunizing power in the 1 per cent vaccine. The total amount of brain material which a mouse received when immunized with a 5 per cent vaccine was 7·5 mg. and 1·5 mg. with 1 per cent vaccine. It was decided to see if 7·5 mg. of brain material was the irreducible minimum for efficient immunization of a mouse or whether that amount of material could be reduced without prejudice to the level of immunity conferred. A 3 per cent vaccine which would give the mouse a total of 4·5 mg. of brain material was therefore tried alongside with the 5 per cent vaccine. The results of the test showed no appreciable difference between the immunity conferred by 4·5 mg. and 7·5 mg. of brain material administered.

At present anti-rabic treatment involves the administration of a large amount of nerve protein. Efforts were therefore made to see if the pure virus could be separated to an appreciable extent, from the nerve material, and a vaccine made as free from nerve protein as possible. The purification of the virus was conducted on the lines of the purification of vaccinia virus by differential centrifugation. The vaccine thus prepared, however, was found to be very deficient in immunizing property.

Experiments were carried out to see if anti-rabic vaccine deteriorated in potency with passage of time, even though stored as it is in cold. In this Institute the vaccine is stored at 4°C. At the centres, however, with the type of refrigerating equipment that they possess, it is likely that the vaccine is stored at much higher temperature. One brew of vaccine stored in the Institute at 4°C. and tested 4 weeks after its date of manufacture was found on a subsequent test made three months after the date of its manufacture to have deteriorated slightly in its immunizing potency. Work is in progress to assess the deterioration at different temperatures and at varying intervals of time, and also to explore any method by which such deterioration can be circumvented.

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3. *Ganapathi, K.*—Action of Sulphanilamide Derivatives in Streptococcal and Pneumococcal Infections in Mice. Ind. J. Med. Res., Vol. XXVIII, No. 4, April 1940, pp. 971–978.

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12. *Gokhale, S. K.*—Blood Urea Clearance in Normal Indians. A study based on the examination of 110 Normal Indian Men. Ind. J. Med. Res., Vol. XXIX, No. 3, July 1941, pp. 627–638.

13. *Lahiri, M. N.*—Studies on Leptospira Ictero-hæmorrhagiae in Rats in Bombay City. Ind. Med. Gaz., Vol. LXXVI, No. 9, September 1941, pp. 536–538.

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S. S. SOKHEY,
Lt.-Col., I.M.S.,
Director, Haffkine Institute.

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